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RELATIVE GROWTH IN THE GARDEN CENTIPEDE[1]

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(Received for publication on August 13, 1943)

For the purpose of studying allometric growth on a highly metamerized form, the garden centipede, *Lithobius forficatus*, was selected. Fifty of each sex were gathered, mounted, and measured with an ocular micrometer. The animals were seriated into size classes on the basis of total body length (see Table 1). The data were plotted

TABLE 1
AVERAGE MEASUREMENTS IN MM. OF THE BODY LENGTH, HEAD LENGTH, AND HEAD WIDTH OF 50 FEMALES AND 50 MALES

Lower limits of size classes	Females				Males			
	Number	Body length	Head length	Head width	Number	Body length	Head length	Head width
11.00	1	12.65	1.32	1.43	1	11.00	1.32	1.38
13.20	3	14.03	1.49	1.61	3	14.83	1.54	1.60
15.40	4	16.40	1.71	1.80	5	16.36	1.69	1.81
17.60	2	18.62	1.87	2.06	2	18.43	1.93	2.06
19.80	4	21.37	2.09	2.26	4	20.79	2.06	2.23
22.00	12	23.09	2.32	2.53	7	23.18	2.29	2.48
24.20	12	25.11	2.48	2.67	14	25.35	2.51	2.65
26.40	10	26.93	2.71	2.88	8	27.38	2.77	3.01
28.60	2	29.54	2.94	3.05	4	29.36	2.89	3.15
30.80					2	31.60	3.03	3.33

logarithmically against body length in conformity with the allometric equation

$$y = bx^k$$

In this equation y represents in turn each of the following measurements: length and width of head and body segments, length of tarsus, tibia and femur of each leg, and total leg length,—the summation of these three segments plus the pretarsus. x in every case represents total body length which is the summation of individual lengths of

[1]From a thesis submitted to the Division of Arts and Sciences of Western Reserve University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author takes this opportunity to express his deepest appreciation for the guidance of Dr. A. H. Hersh.

body segments plus head length. k, the equilibrium constant, gives the slope of a straight line when the data are plotted on a double logarithmic grid. b, the initial growth index, is the y intercept of the line. The values of b in this paper are based on $x = 10$ (see

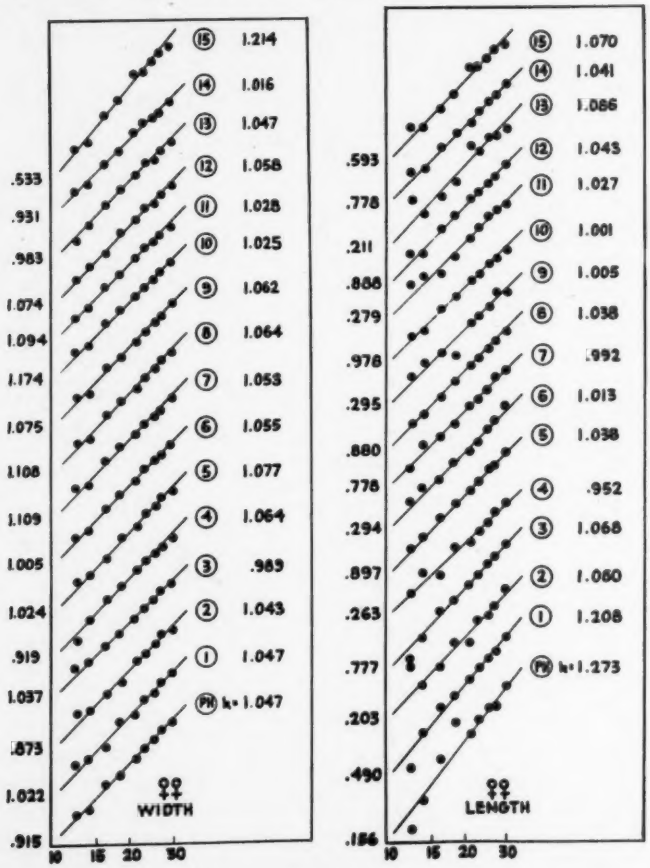

FIGURE 1

SHOWING DATA LINES AND POINTS FOR BODY SEGMENT WIDTH AND LENGTH AGAINST BODY LENGTH IN FEMALES

Values of b are at the left and k values at the right (see text).

Lumer, Anderson, and Hersh, 1942) and, since all original data are in mm, b becomes the value of y when $x = 1$ cm which is very close to the actual length of the smallest specimen, 11 mm. The constants were determined by the regression method of least squares (see Feldstein and Hersh, 1935). Standard errors of k were also determined, the order of magnitude being 0.01.

The general character of the results may be grasped from the accompanying figures. Figure 1 gives the data points and fitted lines for the width and length of the prehensor and the 15 body segments for females. On the right are the k values and on the left the values of b.

It is at once obvious that the k values for both width and length of the segments are close to one, and the curves are approximately parallel. In regard to the values of b for width, there is a decrease from about the tenth segment posteriorly which is an expression in allometric terms of the tapering or streamlining effect of the animal's body. The values of b for length might be interpreted as representing two series, one for the short intersegments (starting with the prehensor and following with the second, fourth, sixth, etc., segments) and one for the longer segments (first, third, fifth, etc.). These two series with few exceptions show postero-anterior relationships up to high points in Segments 9 and 10 for the shorter and longer series, respectively. From these peaks the b values decrease posteriorly. The data for males give substantially the same results.

Figure 2 gives the curves and data points for total leg length and length of segments. Though the legs increase in length from anterior to posterior, it may be seen that the k values decrease from the first to ninth legs but this decrease is not maintained for the more posterior segments. Nevertheless there appears to be a rather flat gradient in the k values for total leg length when only the first and fifteenth legs are compared. Another point of interest lies in a sex difference for while in females the values of k for the fourteenth and fifteenth legs are 1.28 and 0.81, respectively, in males the last two legs have values of 0.72 and 0.79, respectively (see Fig. 3). In any case the gradient is flat. However, when the values of k for the first and fifteenth legs are tested by the method for determining range as outlined by Tippett (see Tippett, 1931) no gradient is revealed. This absence of a marked gradient implies that the shape

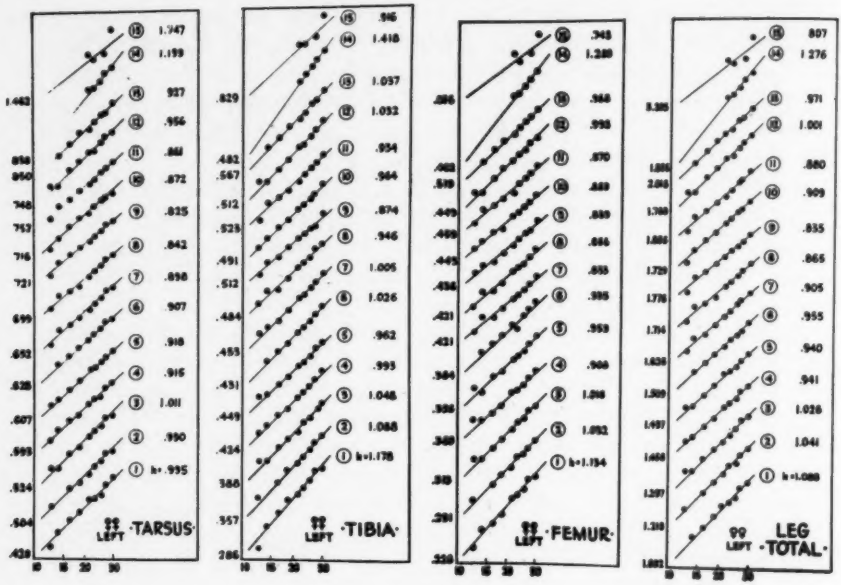

FIGURE 2

SHOWING DATA LINES AND POINTS FOR LEG SEGMENTS AND TOTAL LEG LENGTH AGAINST BODY LENGTH IN FEMALES

Values of b are at the left and k values at the right (see text).

and form of the organism was established before the time represented by the size of the smallest specimen. The values of b may help clarify this point since they show a definite postero-anterior order. Beginning with the first leg, the value of b is 1.00, for the second leg 1.21, for the third 1.30, etc. This is to say that increases in length occur between the first and fifteenth legs. If this is an expression of an underlying growth gradient it is clear that it must have been established prior to the time indicated by the size of the smallest specimen. Thus the values of b bear out the hypothesis that in the main the ontogenetic growth pattern was determined presumably during the embryonic period. Similar relations in b values are found for the legs of Notonecta by Clark and Hersh (1939).

Another case in point exists in the curve of length of rostrum in

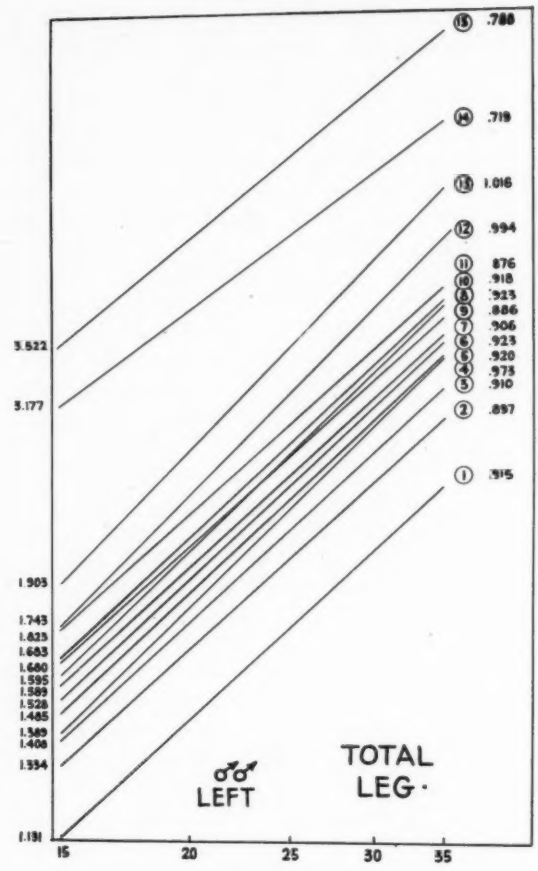

FIGURE 3

SHOWING RELATIVE POSITION OF THE DATA LINES FOR TOTAL LEG LENGTH AGAINST BODY LENGTH IN MALES

Values of *b* are at the left and *k* values at the right.

relation to the body length in the spoonbill catfish, *Polyodon spathula* (Thompson, 1934; see also Hersh, 1941). Here the data show a curve but if the data points representing only later stages of body

length were considered, they could very well be interpreted as conforming to a straight line. Furthermore, if the curve should be extended downward to smaller rostral lengths, k would approach a value close to infinity. Likewise in the centipede, if each of the practically parallel curves for total leg length was extended downward, say to a point where $x = 1$ mm, the approximate size of the egg stage, the total curve resulting would be similar to that of Polyodon. A similar condition obtains in the head of the house wren (see Huggins, 1940).

The foregoing discussion concerned the uncovering of possible gradients along the body axis. Now the gradients within the legs are similarly analyzed. Though in practically all cases the tarsus proves to be longer than the tibia and the tibia longer than the femur, the k values for leg segments (see Fig. 2) present a different picture. It is at once evident that the tibia represents the highest point of growth potential which decreases proximally in the femur and distally in the tarsus. The values of b for the leg segments tend to show a postero-anterior order just as do the total leg length values.

Figure 3 shows the actual and relative positions of the curves for all 15 legs of males, left side only since there is no evidence of asymmetry. The data points are omitted to avoid confusion and to clarify the spatial picture. The abscissa begins somewhat above the size of the smallest specimen to avoid crossing of some of the data lines. It is evident from the figure that here too a flat gradient exists, moreover, the curve for the first leg as well as those for the last two are separated from all the others while the curves for the legs in more central positions are rather crowded.

No figure is presented for data on the length and width of head (see Table 1) but for these dimensions it might be mentioned that the k values for females are practically the same, 0.96 ± 0.01 for length and 0.94 ± 0.01 for width. The corresponding b values are 1.05 and 1.14. In the males the values of k are 0.89 ± 0.01 and 0.91 ± 0.02, and the b values are 1.10 and 1.16 for head length and head width, respectively.

Summary

In the centipede, *Lithobius forficatus,* the length and width of head, length and width of body segments, length of tarsus, tibia,

femur, and total leg length when plotted separately against total body length show conformity to the relative growth equation

$$y = bx^k$$

The values of k are in the neighborhood of unity. This similarity among the k values, in the legs at least, shows a rather flat gradient along the body axis, i.e., the growth pattern shows little change with increasing body size. The similarity among the k values implies that the shape and form of the organism was established, though perhaps not rigidly, before the time represented by the size of the smallest specimen. Within the legs themselves the tibia represents the highest point of growth potential.

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THE RÔLE OF THE AMINO ACIDS AND NUCLEIC ACID COMPONENTS IN DEVELOPMENTAL GROWTH. PART ONE. THE GROWTH OF AN OBELIA HYDRANTH. CHAPTER ONE. DESCRIPTION OF OBELIA AND ITS GROWTH

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(Received for publication on August 23, 1943)

> Knowledge, then is not knowledge of appearances, but appearances are knowledge of substance when they are taken for signs of it.—
> Santayana: The Unknowable

CHOICE OF ORGANISM

Organisms are the tools wherewith we uncover the interdependencies of living. Some suit one line of inquiry; others another. As the skilled worker chooses the tool best suited to the work at hand; so does the investigator select the organism which will bring to light the information he is seeking. So concern here is not with organism as organism; but with organism as test material suitable for evaluation of the interdependencies of growth.

As stated in the Introduction (14) present interest is in the relations between the naturally occurring amino acids and nucleic acid components, and growth. And growth is taken to be compounded of the activity elements of Initiation, Proliferation, Differentiation, Organization, and Constructive Substance Increase; with which is intercalated the plasergic activity of Maintenance (11:13).

The approach to resolution of these interpendencies boils down then to a determination of the effect of each separate compound on each separate activity. Specifically—What is the effect of histidine on initiation? On proliferation? On differentiation? On organization? On constructive substance increase? On maintenance? And so on similarly for each of the naturally occurring nitrogenous tissue constituents of general distribution.

It has been customary in growth studies to measure the expression of but one, or at most two, activities in any given organism. Most

organisms present only one or two activities which can be measured distinctively and accurately during the usual period of observation. Moreover, investigators seem to be more interested in growth as an entity than as a compound of activities. Some think increase in number is its chief expression (19). Others consider mass or size increase is its only component (2). And to others the activities of initiation, differentiation, and organization are not growth at all (13).

Those who consider all growth is proliferation, measure increase in number only, and choose their organisms accordingly. Those who consider all growth is comprised in weight change, measure this or dimension only, and select organisms on this basis. And so on similarly. Such procedure does not allow determination of whether or not a given compound is specifically important to a given growth activity. Because—

If it be granted that growth is a compound of interdependent activities, then it can happen that induction of change in one by a given compound may produce change in expression of some other. And this could be of sufficient magnitude and nature to prevent conclusion as to which of the two measured activities is directly subject to the given compound. For example—

When both cell specialization and cell increase in number are enhanced in mouse skin on application of sulfhydryl (8:16) it is impossible to tell from this alone whether it is differentiation or proliferation which is primarily affected. It is only because other material shows sulfhydryl to be specific to cell increase in number (7:9) that the differentiation response can be taken to be secondary.

This is not the only type of potentiality for uncertainty. For when only one activity is measured or evident, no proof can be had that this and this alone is the one directly affected. For example—

The rat is widey used for growth studies. Changes in its weight are taken as indices of the significance of compounds to growth. But weight gain is simply a mark of constructive substance increase; only that and nothing more. Even so, such may not be constructive substance increase. In some cases some of this is water (5:18). And fat deposition in aging animals (4) is not growth but storage. These facts alone show the need for caution when dealing with weight change and growth.

When young rats are fed histidine-deficient diets they fail to gain

weight (17). The conclusion has been that the compound is directly concerned in growth. But the removal of histidine from the diet of older rats where growth is negligible causes them to lose weight (6). Loss of weight can be caused by failure of maintenance. So it has been concluded that histidine is directly concerned in this non-growth activity.

Since maintenance is a factor of living in young as in old rats; since constructive substance increase is subtended by maintenance (14); it follows that failure of maintenance may induce failure of constructive substance increase. So then it could be that the failure of young rats to gain weight on histidine-deficient diets is not due to any direct connection of this amino acid with constructive substance increase at all; but simply to absence of its sustaining influence on maintenance. It happens that histidine is specific to maintenance and not to constructive substance increase (10:12). But the answer was not obtained from weight change alone, nor could it have been.

These examples suffice to show that the use of an organism in which but one, or even two, growth activities are separably measurable can lead to uncertainty and confusion in so far as interpretation of specificity of action of a given compound on a given activity is concerned.

No inference is to be taken that such organisms are unsuitable for any growth study at all. The use of such has laid foundations and suggested paths by which growth may be integrated with living as a whole. While they may not be suitable for indicating the existence of specific relationships, they are suitable for confirmation of such when this is found to exist in an organism where uncertainty is minimal. They thus extend the finding from the particular towards the general. And this of course strengthens the foundation for conclusion.

With these things in mind I came to the conclusion it would be advisable to use an organism with the following characteristics:

1. *It should exhibit ALL the basic growth activities in full expression during the period of observation.* Only with such can be had that simultaneous exposure of all activities to any given compound which is necessary to determination of differential reaction.

2. *It should exhibit each activity in such fashion its expression*

is distinctive and separable from that of the others. Otherwise no indication can be had the reaction is specific and unmixed.

3. *It should exhibit the expression of each activity in such manner the extent thereof can be separately estimated with reasonable accuracy.*

4. *It should customarily derive its nitrogenous subsistence from pre-formed amino acids assimilated from the external environment.* Since it was planned to present pre-formed amino acids it seemed better to use an organism which feeds on these than one which ordinarily does not assimilate its nitrogen in this form. By so doing the possibility that adjustments of assimilatory processes to compounds foreign thereto might interfere with their participation in growth. This eliminates plants from the primary explorations. It does not mean that plants are not suitable or even necessary test material for the inquiry. They are. But their best use is as checks on results obtained with animals. They must of course be used before sure conclusion can be had that any specificity found in animals is not peculiar thereto; but is expression of a basic and universal relation.

5. *The organism should be capable of withstanding laboratory culture without serious dislocation of its usual growth progression.*

6. *The organism should be available in sufficient abundance for extended experimentation.*

Whether or not an organism existed which would meet these requirements was generally unknown. The facility with which marine forms lend themselves to physiological experimentation; their wide variety; the relative simplicity of their growth expressions; and their abundance suggested that among such might be found one which would be suitable.

This brought to a head the need for a place to work where there was no long season of high temperature and humidity, and a place where the water supply was uncontaminated with chlorine and ammonia, or the atmosphere with chemical fumes from nearby industries. Growth research of the kind proposed cannot be done under such conditions.

So the Marine Experimental Station of the Lankenau Hospital Research Institute was established in the Spring of 1930 at North Truro on Cape Cod, Massachusetts, through the generosity of Mr.

August Bein and Miss Amelia Bein of Philadelphia, and the Capt. L. D. Baker Estate of Boston and Wellfleet.

The location was chosen because it afforded in easy accessibility an abundance of marine and fresh-water fauna and flora in wide variety, together with sea and fresh water absolutely uncontaminated by sewage or manufacturing wastes, and an atmosphere free from smoke or other growth distorting factors. The place was sufficiently isolated to minimize interruptions of the work by visitations from the merely curious. But it was close enough to Woods Hole to allow trips thereto when consultation of the library provided by the M. B. L. was indicated. And of course the climate and environment were not displeasing.

With these facilities available, search for a suitable organism was begun. Having no formal acquaintance with systematic zoölogy the mind was not clotted with preconceptions, but was free to consider each specimen collected as a growing thing and not as a museum piece.

The obvious procedure was followed, namely: to collect one after another specimens of each kind of littoral organism, bring them to the laboratory in a plentiful supply of fresh sea-water, bed them down in the components of their natural habitat, and study the manner of their growth.

The first season failed to uncover what was wanted. It did engender a respect for the sensitivity to handling of the little animals. It did teach how they were not to be treated if their integrity was to be maintained. Fewer controversies would arise if those who work with such material would learn this lesson.

The 1931 season brought to the laboratory an organism which at first view under the microscope seemed to be nothing more than an assembly of different-shaped knobs and flower-like objects on a stalk. When it was found after 24 hours of culture in frequent changes of fresh sea-water that some knobs had changed to the flower-like structures, and that it was possible to distinguish one knob shape from another with surety, it was evident that here was an animal which might serve our purpose. With the help of our *vade mecums* the organism was identified as the colonial-living form of a marine hydromedusa belonging to the genus *Obelia* (1:3). A search of the available literature gave no indication that anybody had bothered to report the intriguing differences in knob shape or what they might signify

in terms of growth. So the summer was spent in following the changes and evaluating them in terms of growth activity expression. The results of this and successive studies are the subject of this chapter.

History and Distribution

In as much as Mayer in his compendium of the literature (86) cites Linnaeus (81:82:83) it may be inferred that this system makes record of what is now known as Obelia. The genus, however, was not founded until fifty years later. In 1809 Peron and Lesueur (94) so designated a form which had previously been described by Slabber in 1781 under the name of Medusae Marina (97). From then on for another fifty years there was considerable confusion. Forbes included both Obelia and Phialidium in his genus Thaumantis (129), and Gegenbauer mixed it in with Eucope (59:60). Finally in 1857 McCrady restored the name to organisms having the generic characteristics exhibited by O. Commissuralis (87) and the genus as it is known today was re-established. Between that time and 1910 claims have been made for the existence of some twenty-eight species (86). More of this anon.

With the assistance of Mrs. Irene Corey Diller, Secretary to The Editors of Growth; Mrs. T. H. Montgomery, Librarian to the Marine Biological Laboratory, Woods Hole; and her assistant Miss Deborah Lawrence, a bibliography has been compiled from Mayer, the *Zoölogical Record,* and *Biological Abstracts.* While attempt has been made to give a complete listing, it is probable that some papers have been omitted. Some were not available for checking. For this reason some references are incomplete. The compilation, faulty though it may be, does none the less provide a guide to most of the literature.

Despite the fact some texts give the impression the genus Obelia is only found along the northern Atlantic seaboard, many reports attest to its ubiquitous existence in all the seven seas. It has been reported from the Gulf Stream (21:45), the Caribbean (85), and the Middle (48) and South Atlantic (36). It has been collected in the waters of the Aleutians (43) and Alaska (44:92); and southward along the West Coast from Puget Sound (41) and Vancouver Island (53), past San Diego (108:109) to Central America (46).

It lives in the Sea of Japan (84:110-111), the China Sea (64:80), and off the Philippines (93). It is known in the South Pacific (68). the Tasmanian Sea (23), and the Antarctic Ocean (76). It is not absent from the Bay of Bengal (89) nor the Indian Ocean (62). Nor is it unknown in the Adriatic (91) and the Mediterranean (98). Clearly the family is quite a traveller and colonizer.

From all reports the genus seems more abundant in temperate than tropic waters (86). This has been attributed to the fact that the plants to which it anchors itself are less abundant in tropical shallow waters. On the other hand it does attach to Balanus, Mytilus, and other shells, as well as to wood-piling. These are present on tropic as well as temperate shores. Adequate bases for its establishment would therefore seem to be afforded in both locations.

Observations over several years suggest that temperature could be a factor in this unequal distribution. It has been noticed every year that as the waters warm up during the summer, an increasing percentage of colonies have to be discarded as unsuited for growth studies because of frailty. Contributory in the late summer may be the drying out effect of the long warm sunny days on the algae and rocks to which the communities are attached. As these become warmed up with advancing season they lose their moisture at low tide sooner than in the spring and early summer. Thus the water adherent to the colonies tends to evaporate to greater degree and its salt concentration is increased. Such a shift could be a deterrent to flourishing growth. For Obelia is a littoral organism unaccustomed to continuous exposure to sea-water of high salinity.

Laboratory experience sustains and strengthens the temperature concept. Not a season has passed but that one or more experiments have had to be discarded because of the *zu grunde gehen* of colonies on hot days. And this despite frequent changes into culture solutions of fresh sea-water. Never were such discards necessary when the culture solutions were cool or cold. Later Sections will give statistical demonstration of this relation together with an approximation of the temperature gradient.

These observations make it pretty certain that exposure of Obelia colonies to water warmed to summer heat may be detrimental to their vigor. It thus seems probable that the lesser abundance of Obelia

in tropical waters could be due to higher temperature as well as to deficiency in anchorage material.

Speciation and Description of Colonies

The identification of the particular species of Obelia used here is based on Mayer (86). While the medusae were not used, his estimate will clarify the situation. He writes: "At present the natural classification of species of such genera as Obelia . . . is impossible, and the Linnean system is inadequate to the task of expressing their actual relationships. Indeed, with the exception of the sponges and corals, there is no phylum of the animal kingdom more difficult to classify than the medusae." "Many of the medusae are modified more or less profoundly by environmental conditions, and this gives rise to numerous local races, the determination of the relationships between which is all but impossible in the present state of our knowledge."

The same uncertainty exists with respect to hydroid stocks. Differences exist, ". . . yet some of these differences . . . are not of specific value, but are probably due to the influence of environment upon the individual, such as favorable or unfavorable conditions of growth, geographical situation, depth and temperature of water, light, etc. It is also true that some of the observed differences in the hydroid stocks are probably of good specific value. . . ." "Undoubtedly many 'species' have been founded on mere geographical or environmental characters of a racial rather than specific nature, and hopeless confusion has been introduced into the synonymy."

Under such conditions all one can do is to identify the organism according to its best fit with available descriptions; give it the name accredited thereto by the authority whose opinions coincide with the observations, and leave the quarreling to the systematists. The Obelia used here comes closest to the species designated by Allman (117) as geniculata. The following description is taken from Mayer (86). The points of agreement of our organism with that description are italicized.

"The stems of the hydroid arise from a creeping stolon usually found growing upon sea-weed near low water mark. They are rarely over 25 mm., are zig-zag, and rarely branch. *Stem is jointed at each of the flexures and thickened immediately below* them so as to form

a series of knees or rests from which the pedicels arise. *The pedicels are short and usually consist of about 4 to 6 rings. They are inclined at an angle of about 30° with main stem and taper outwardly. The hydrothecae are short, being nearly as wide at the aperture as they are long. They are nearly conical with slightly bulging sides and a smooth upper edge. The reproductive calycles are borne upon a short stalk consisting of 3 to 4 rings. They are club-shaped and taper gradually from base to outer end where they are widest. A short tubular orifice arises from the distal summit of the calycles. Each gonangium bears numerous medusa buds." "Obelia geniculata may be* at once *recognized as the creeping hydroid with short,* unbranched, *erect, zig-zag stems, growing* in great quantities *over the fronds of Laminaria.* Hardly a piece of kelp is free from this hydroid. *It is a conspicuous object on account of its light coloration standing out in contrast with the dark brown of the kelp."*

This then is the frame within which our organism is contained. Some of the characteristics are essentially invariable. Others vary; but within limits. Analysis and further description may be useful for later speciation.

Invariable is the origin from a creeping stolon; the limitation of hydrocaulus length to 25 mm.; the presence of jointed and thickened flexures; the short pedicels with but 4 or 6 annulations; the 30° angle of their inclination outward from the hydrocaulus; their outward tapering; the smooth rim of the hydrothecae; and the gonangia with their short annulated stalks and medusae.

Variable is the anchorage which may be on Balanus, Mytilus, bare rock or wood, as well as on Fucus and other algae. The zig-zaggedness is rarely marked. It is more a matter of pedicular geniculation than alternation of direction of deviation of the main hydrocaulus from linearity.

The appearance and degree of geniculation is correlatable with age. Colonies in culture exhibit apical growth with production of new hydranth-bearing pedicels. It may be inferred that population increase of single colonies is similarly accomplished in nature. So the apical pedicels are younger than the basal. In colonies as collected there is increase in structural differentiation from apex to base; i.e., geniculation is greatest in the basal hydrocaulus and diminishes apically. Geniculation is also minimal in the new pedicels arising

in colonies in culture. That is to say, the newer the pedicel the less the jointedness and sub-flexure; the older the pedicel the greater the geniculation. Such being so it could happen that colonies or fragments of colonies of meagre geniculation are but young specimens of a species in which more marked geniculation is a product of aging. Acquaintance with this possibility might eliminate one factor for confusion in speciation and identification in the genus.

Age changes also occur in the perisarcal chitin of the pedicular jointure with the hydrocaulus. The chitin of the newer pedicels is thin, essentially colorless, water-clear, and flexible. That of the older basal pedicels is thicker, brownish-yellow, less translucent, and more rigid. The elasticity of the young chitin is demonstrated in cinematic record of the developing pedicel, where the outgrowth is seen to be stretched distally by recurrent surges of hydroplasmic pressure. Such stretchability is not encountered in the older structures.

Although these age changes in amount and character of the chitin run parallel with the progress of geniculation, no evidence justifying the conclusion of factorial interdependence is had. Geniculation can be taken to be the result of genetic direction of differential deposition through the exercises of the basic growth activities of constructive substance increase and cell specialization. The changes in chitin characteristics can be taken to be the natural result of entropy and polymerization inherent in material of this chemical constitution. In other words, age changes in chitin cannot be taken as responsible for geniculation; nor can geniculation be taken as responsible for the age changes in chitin. Differences in amount and characteristics are by themselves clearly unsure indices of species specificity.

In nature the pedicels are alternating. In culture it sometimes happens that a new one arises at the junction of a pre-existing element with the hydrocaulus.

The distance between the alternating pedicels is essentially uniform.

The variety of *O. geniculata* found here is usually branched. Even that found growing on Fucus. The usual number of branches is two. There may be three; but rarely four. These all occur in the basal region; rarely if ever in the middle or apical. In laboratory culture, these branches, like the main hydrocaulus, exhibit apical growth. They also produce new hydranth-bearing pedicels. These growth activities will shortly be described in detail.

Despite the symmetrical drawings given in Mayer and elsewhere, there is considerable natural variation in hydrothecal shape and ratio of length to width at aperture.

Now the cinematic record of hydranth growth shows that the completely organized animal has to break through a distal chitinous membrane of the hydrotheca before emergence as a functioning animal. It accomplishes this break-through by a succession of remarkably vigorous out-thrusts.

The cinematic record also shows that the chitinous hydrotheca is stretched lengthwise by these surges of the within-contained animal; and that this retracts towards its original state during their intermission. As a result the hydrotheca may be much longer than wide at one time, and almost as wide as long at another. Sometimes it is almost truly conical; and sometimes its sides are perceptibly bulged. It all depends on whether the animal within is expanded or relaxed.

These facts mean: first that the ability of the hydrotheca to take different shapes and proportions resides to considerable degree in the elasticity of its chitin; and second, that variations in hydrothecal shape and proportions may be largely conditioned by pressure differentials. The limits of course are genetically determined.

Such being the case the environment affords many opportunities for production of colonies which differ in hydrothecal shape and proportions; but which nonetheless may belong to the same species.

Thus, littoral specimens collected after long exposure to the water pressure of high tide could differ from those obtained after long exposure of low, where such pressure is absent. And the same could be true of colonies collected after a period of exposure to vigorous wave pressure and those obtained after a long calm.

Such trends have been noticed by us; but the direction and maintenance of difference has not been recorded. Furthermore, specimens collected from places where tidal current activity past their anchorage is consistently vigorous, tend to differ from those found in more protected habitats. In such cases it could happen that the direction of difference would be constant and give rise to the idea that the two lots belonged to different species. But such conclusion might not be justified.

Although I have no other evidence than what my eyes afford, I am convinced the chitin of Obelia undergoes a progressive decrease in

elasticity as the organism grows older. I conclude from this that the chemical processes of entropy and polymerization tend to bring the hydrothecal and other chitin to a state of relative rigidity where changes in shape are not readily effected. In such a state, a shape and proportion produced by continued pressure conditions peculiar to environment during the time the chitin is more amenable to change, might well become relatively fixed and unreversible. Such a constantly occurring difference would be environmental and not species specific.

Mayer in his descriptive tabulation makes note of hydrocaulal color as if this has sometimes been taken as a mark of species distinction.

It has been observed here that whereas the geniculata colonies are either colorless or a transparent pale yellowish in early Spring, many tend to become brown or black as Summer progresses. This is a seasonal change, for the proportion of blackened hydrocauli increases as the season advances. By mid-September it is difficult, if not impossible, to find uncolored specimens. But as Winter comes the trend is reversed and by Spring again there are essentially no discolored stalks to be found at all.

Examination of mid-season hydrocauli under low power might give the impression that the colored specimens are inherently different from the uncolored. But careful study under higher power reveals the discoloration is largely due to apposition to the hydrocauli of brown and red algae and debris.

Because of this, and because the seasonal change in color is correlatable with the seasonal change in plankton population, the logical conclusion is that in many instances coloration may be seasonal and not species specific.

The discoloration also varies with locality. Specimens from sandy open shores have less than those from places where tide water drains off from muddy marshes. In the latter there is often true darkening of the hydrocaulal substance itself. The number of such and the intensity of darkening progressively increases as the late season advances. That is to say, as the decomposition processes taking place in the marshes increase with advancing late season, so does the virtual-darkening of the geniculata hydrocauli exposed to the water draining therefrom increase.

This correlation makes probable that this hydrocaulal discoloration

is a result either of ingestion and absorption of human-like substances from such water, or of reaction of H_2S contained therein with heavy-metal ions, rather than a result of any inherent specificity in pigment metabolism. It is an environment and not a species distinguishing characteristic.

These things being so, it is clear that in our locality at least, season and environment are definite factors in Obelia hydrocaulal colorations. It is not improbable that such is the case elsewhere.

These observations as a whole, then, are pretty good support for Mayer's thesis that many differences in hydroid stock taken as of specific value, are not such at all—"but are probably due to the influence of the environment upon the individual, such as favorable or unfavorable conditions of growth geographical situation, depth and temperature of water, light, etc." In fact I should say that they establish the point of view as correct beyond any reasonable doubt, and justify Mayer in labelling his tabulation as a "General Classification of the *So-Called* Species of Obelia" (Italics mine). These findings also suggest that the "hopeless confusion (which) has been introduced into the (Obelia) synonymy" may stem from ignoring or ignorance—on the part of the speciators—of the possibility that structural and other variation may be induced by physiological and chemical reactions to environmental changes.

The evidence for this opinion is the paucity of data in the literature from which such evaluations can be made; the absence of record of such correlations; and the apparent utilization for decision of fragmentary and moribund colonies like those pictured by Mayer in Figures 25, 29, and 31. These are no healthy viable specimens, for such never present an array of empty dislocated hydrothecae. It is only when colonies are mistreated by nature or by man that such-like specimens appear. They are not representative, and their use as such suggests a disdain not only for the processes which produce the marks of speciation, but also for the processes which contribute to their variation.

While our observations in this direction were incidental to our need for healthy colonies, the conclusions derived therefrom have no weak foundation. For we have examined tri-weekly collections of hundreds of Obelia geniculata colonies from the same anchorages from April to mid-September for seven or eight years; and have gotten them

therefrom at intervals during some winters. And we have seen how structural and other variation may be induced therein, not only by time, tide, temperature, season, and locality, but also by transfer from natural habitat to laboratory culture. Later sections will give statistical demonstration of the influence of these factors on growth. For the present it is sufficient to suggest that taxonomy might find physiology a useful adjunct in diminishing confusion in speciation.

It is worth noting that the medusae-bearing gonangia of our Obelia geniculata are almost exclusively located in the pedicular crotches of the basal hydrocaulus; never in those of apical regions, and rarely, if ever, in those of the branches.

Growth of Obelia: Colonies

Obelia colonies are said to derive from ciliated embryos which have fixed themselves upon an appropriate littoral substrate. These embryos are said to be the product of fertilized ova originating in free swimming medusae which themselves are the product of other colonies (260:261).

Nobody has written much of how the colony grows once it is established, beyond stating that its popualtion is increased by continued buddng from the hydrocaulus (178:239).

The concept of colonial growth presented here is derived from two sets of related observations. The first consists of observations on the kinds of structures produced by colonies in laboratory culture. The second on observations of communities of colonies *in situ*.

Now it seems logical that a decent regard for the integrity of living test material in the conduct of experiment should yield results of greater reliability than a procedure in which the amenities are carelessly disregarded. Therefore we have attempted to so handle our organisms from the time of collection, through their period of culture, to the recording of their reactions, that they be subject to as little insult as is possible consistent with the needs and exigencies of experimentation.

Obelia is an organism of great beauty and some delicacy. When colonies are collected with due respect for their fragility, and brought to the laboratory in an abundant supply of fresh sea-water, they will thrive and grow when cut cleanly from their anchorage, if cultured singly in 35-40 c.c. of freshly collected sea-water, provided this is

frequently renewed. Under such conditions a colony of from 25 to 30 animals, a few gonangia, and one or two branches, will produce one or all of the following structures as additions to its initially present state.

New hydranths arise by budding from the apical hydrocaulus, be this the main or branched stalk; and already present immature hydranths develop into functioning organisms. Under conditions which have not yet been analyzed new hydranths may also bud out from the region of pedicular jointure of already present animals with the hydrocaulus.

Colonial growth, other than by increase in hydranth population and hydrocaulus extension is exhibited in colonies in culture. Thus new gonangia bud out from the region of hydranth pedicular jointure with the hydrocaulus in the basal reaches; and already present gonangia develop to the stage where their therein growing medusae reach the free-swimming state and are liberated into the surrounding culture medium. And a stolon grows out from the cut end of the hydrocaulus.

This stolon can attach itself so firmly to the bottom of the culture dish that the colony can hardly be removed therefrom without destruction of the new rooting. It can be imagined that this property of new stolon formation is utilized in nature for colonial reimplantation in instances where colonies have been separated from their attachment by storm or other agent. If this be so the property may be a factor in colony persistence.

Colony growth in culture is thus seen to be effected by the addition of new hydranths, hydrocaulal extension, new gonangia, and a new stolon.

Now Obelia colonies live in communities whether anchored on Fucus, Mytilus, or Balanus. The colonies comprising such communities vary greatly in size and population. They may range from small colonies carrying 3 to 5 hydranths and but 5 to 10 mm. in length, through medium ones with 12 to 18, to populous members having from 30 to 60 and a full 25 mm. extension. The smaller colonies have neither branches nor gonangia. Such are first found in the medium-sized members of the community, and their number and complexity is greater in the colonies of greater hydranth population. Furthermore, immature as well as mature hydranths and gonangia are present. Correlating these facts with the observations of

colonies in laboratory culture the following concept of colony growth in natural habitat seems close to verity.

The colony grows in size by distal extension of the hydrocaulal stalk, and in population by producton therefrom of buds which develop into hydranths.

Hydranth budding does not occur helter-skelter along the hydrocaulus. It takes place therefrom just posterior to the pedicel of the most distal member. This most distal member is at first a prolongation of the hydrocaulus; appearing at its apex. It is not a branching off therefrom at this time. Shortly, however, a bud appears just below. This now becomes the prolongation of the hydrocaulus and at its tip a new hydranth develops. As a result the animal which previously was the terminal member of the colony becomes a pedicular-supported off-shoot, and is no longer a prolongation of the main stem. And so on similarly with successive additions coming off alternately on opposite sides of the stalk.

Branching apparently originates by a similar process taking place from the pedicel of a basal hydranth. The stimulus to branching is unknown to us. The studies of Davenport (185) and Lund (244-249) on hydranth regeneration, suggest that injury may be a factor. Regardless of its origin, once a branch is started, new hydranths are added as to the central stalk.

Although the colony is a colony of hydranths, and is characterized thereby, it also produces gonangia within which the gamete-bearing medusae develop. These gonangia bud out from the basal hydrocaulus in the region of hydranth pedicels. They do not appear until the colony is well developed. They are few in number in comparison with the hydranths. The impression is had that their coming is season-conditioned. But we have no statistical proof as yet. Elmhirst (187) postulates a lunar periodicity. But here again statistical proof is not available.

The stolon also is part of the colony. It grows in pace with the colony as a whole. Whether it ever stops growing while the colony continues to increase in size and population is unknown to us. Nor do we know whether single stolons arising from single embryos merge to form a net-work whereby hydroplasmic inter-communication of colonies in community is afforded. These matters, however, are not particularly pertinent to present interest. What is pertinent is the

manner in which the colony gets material for its substance increase.

Once mature hydranths are present, these take care of the colony. But after storms, colonies are found completely decimated of hydranths, yet such may put out new buds. Where does the substance used here come from? Not from feeding hydranths of the damaged colony for there are none. Does it come from other less damaged and hydranth-bearing colonies through hydroplasmic inter-communication via a stolon network? Is the material of the new growth derived from the tissues of the damaged colony itself through autolysis? Or does it come from absorption by the stolon?

Since such colonies in culture seem to become thinner, paler, and more transparent as new hydranth buds appear; and since autolysis of the substance of worn-out hydranths does occur, it is possible some if not all the material does come from tissues of the damaged colony itself.

Once the colony is established as an assemblage of mature hydranths, however, the substance for increase is provided by the hydranths to great if not exclusive extent. This has been demonstrated by observation and experiment.

Structurally the animal consists of tentacles; a terminal fleshy orifice or mouth; and a gastric cavity or stomach connected with the mouth by what may be taken as an oesophagus. The stomach contents are continuous with the pedicular hydroplasm, and through this with the hydroplasm of the colony as a whole (Plate 6, Figure 1).

Cinematic record shows tentacles, mouth, oesophagus, and stomach engaged in lively coördinated activity. The tentacles sweep the surrounding sea-water and carry what sticks to them to the mouth. Single tentacles gracefully bend inwards towards the mouth, the mouth reaches out, expands cup-like, the tentacle gently draws itself across the lip and even inserts its tip within the cup as withdrawal proceeds, and then the mouth recedes and slowly contracts. The mouth is now a ball and now a cup on a long thin stalk; again it may be a wide cup on a short thick stalk; the oesophagus. The stomach is at one time a narrow cylinder; at another a rounded sac.

Observations show particles bobbing around in the gastric cavity; passing therefrom into the hydroplasm of the pedicel; and travelling therein throughout the colony. These are shown by experiment to

consist in large measure of food particles undergoing digestion, since they give test for free amino acids (214).

Observations also show that the hydroplasm streams out into the growing immature structures and experiment shows that amino acids added to the surrounding sea-water are taken in by oral ingestion; concentrated in mouth and stomach; and passed therefrom into the hydroplasm which carries them throughout the colony where they are re-concentrated in the actively growing and immature structures (182:214).

All this is indubitable evidence that the colony gets material for its substance increase through hydranth feeding activity.

To summarize: Correlation of structural, physiological, and *in vivo* chemical evidence establishes beyond question that Obelia follows classic procedure with respect to ingestion, preparation, distribution, concentration, and utilization of amino acid building materials for growth. Protein is taken in by the hydranths and broken down into its constituent amino acids in a digestive system; it is distributed throughout the colony as amino acids in a vascular system; it is concentrated as amino acids in tissue where growth is taking place; and these are incorporated therein as new protein is built into the structures.

One more note and we are free of colony growth for the present. There appears to be a diurnal variation in feeding which is associated with tidal rhythm. Colonies collected in the early morning at low tide feed up to 10 o'clock or thereabouts. Then they go into a period of rest with tentacles withdrawn into the hydrothecae. Along about 4 o'clock in the afternoon tentacles are again put out and feeding is resumed. Attempts to prolong feeding time by changes in temperature or illumination were futile. Change into fresh sea-water sometimes gave a transitory stimulus to feeding; but this was uncertain. Tests made of amino acid distribution and localization made during and shortly after feeding were strong and consistent. Those made 3 or 4 hours after feeding were weak and variable. It is evident that ingestion, digestion, and utilization of materials for growth is not continuous; but intermittent. The parallelism between the nutritive mechanisms of Obelia and those of the so-called higher animals is edifying.

It has now been demonstrated that O. geniculata satisfies two of

the basic requirements for suitability as test material in exploration of the rôle of amino acids and nucleic acid components in growth, viz. —it is available in sufficient abundance for extensive experimentation, and it derives its subsistence from pre-formed amino acids assimilated from the external environment.

Under the next heading it is proposed to complete the demonstration of suitability by showing the animal exhibits all the basic growth activities in full expression in such fashion that each is distinctively separable from the others; that the expression of each activity can be measured with reasonable accuracy; and that growth progression occurs in laboratory culture without serious distortion from the usual.

Obelia Growth: Hydranths

It is probable that the first and only notation of the structural changes incident to Obelia hydranth growth up to 1933 was made by Brooks in 1882 (178). This worker with singular perspicacity not only had his students make sketches of the several distinctive stages, but also called their attention to the fact that food is carried into the animal by the hydroplasm long before it has a mouth of its own. My only excuse for not having given credit to this great pioneer in previous papers is that his description was buried in a Text-Book of Invertebrate Zoölogy, which did not come into my hands until recently. His recognition that Obelia is a living, growing animal suitable for study and demonstration of the principles of growth and physiology, at a time when others were almost exclusively concerned with speciation, stamps him as head and shoulders above his predecessors, his contemporaries, and even his successors, not only in the field of the marine hydroids, but also in physiological zoölogy in general. He was fifty years ahead of his time. And he has suffered the fate of those who run too far ahead of the pack. Perhaps this quite inadequate tribute will bring re-recognition of his breadth of horizon.

If one should compare my first description of the life-cycle of an Obelia hydranth (192) with those which have followed, and also the unfolding of the concept of growth which this has stimulated (14:192-235) one will be struck by the essential verity of the Paretan (256) principle that "logico-experimental theories are in a state of continuous development and they advance by successive approximations."

Even that which is now set down is also but an approximation. Only time can tell whether its essence is of more than transitory utility.

The growth of an Obelia hydranth is best illuminated by two sets of photographs. One of a live animal as it develops from an apical hydrocaulus, and one of fixed and stained specimens of various stages.

The pictures of the living animal are taken from a cinematic record of the growth of a single individual from its beginning to its maturity. They are thus slices from a continuum. As such they are unequivocal evidence that the sequence of growth progression is as stated.

As far as I know this is the first and only complete photographic record of the continuous growth of one and the same multicellular organism from beginning to end. The film was taken under the supervision of Professor R. Lee Walp of Marietta College. Shots were made every 15 seconds for 72 hours by the laboratory Staff working in couples for 4-hour shifts. The film was exposed under rather primitive conditions. Difficulties of illumination arising from varying electric current, and shifts in position of the growing animal made focusing not all that could be desired. Nonetheless the pictures are adequate for the purpose.

The animal pictured here arises from the hydrocaulus just posterior to the terminal hydranth. The first indication that such is beginning is the formation of a plateau within the hydrocaulus as pictured in Plate 1, Figure 1. It is from such that a new organism buds out as seen in Plate 1, Figure 2. The plateau is therefore the anlage of the new animal. Its formation is a preparation for new hydranth production. The cinematic record shows very vigorous hydroplasmic activity localized in the vicinity of the plateau as it is forming and prior to emergence of the bud. The contrast between the localized activity and the quiescence of adjacent regions is evidence enough that here there is shift from a state of non-growth to a state of growth beginning. This is an expression of what is called the Initiation phase of growth; or the phase where processes preparatory of new growth production take over from processes which previously were largely concerned in maintenance (11:14).

The true student will not be misled into the assumption that Initiation is a figment of the imagination by the fact that anlagen formation is accomplished by cell increase in number, cell specialization, and

cell increase in mass. He will realize that before these can occur there must take place within the hydrocaulal cells a change in kind of activity from that of maintenance or non-growth, to a kind wherefrom cell increase in number, cell specialization, and cell increase in size would take place. Thus he will know that the anlage as he sees it is a true mark of the basic growth activity of Initiation, and that the number of anlagen which appear in a colony during a given period is a measure of the expression of this growth activity during that period.

Once preparation has established the anlage, the animal buds out from the hydrocaulus as pictured in Plate 1, Figure 2. The hydrocaulal perisarc is as found retouched in Figure 1.

One glance at Plate 5, Figure 1, will show that bud growth is dominated by cell increase in number.

Comparison of Figure 1 and Figure 2 of Plate 1 will make clear the clean-cut structural distinction between the smooth unbulging surface beneath which lies the anlage, and the convex bump which is the bud. It is a case of bud or no-bud. Thus the expression of Proliferation is separable from that of Initiation.

First from the bud comes the annulated pedicel as shown in Plate 1, Figure 3. The elongation is accomplished by cell increase in number, aided and abetted by mechanical outward stretching. The evidence is this.

It has been found that sulfhydryl is concentrated where cells are dividing, and that this naturally occurring chemical group acts to forward the basic growth activity of proliferation (7:9). Tests for SH distribution in living growing Obelia pedicels made under the microscope show that the group is "—concentrated right in the location wherefrom the new growth is springing. That as this proceeds to form a bud and grow a pedicel, the intensity of concentration increases in the most distal—growing part, and diminishes proximally therefrom *to leave entirely* the region of origin in the hydrocaulus" (182).

This is chemical evidence, first that proliferation is actively contributing to pedicular growth, and second that the seat of this contribution is largely located in the apical structure or bud.

The continuous cinematic record of pedicular growth shows that from the beginning the pedicel is subjected to distal stretching by

recurrent forceful pulsations of its therein contained hydroplasm. These pulsations emanate from peristaltic-like activity within the main stem or hydrocaulus. With each building up of internal pressure the structure stretches outward, to recede towards its former length as the pressure subsides. Whether waves of increased proliferation activity alternate or are coterminous with these recurrent outward thrusts is unknown. All that is sure is that the accomplishment of pedicular elongation is rhythmical in nature; that the dominant growth activity is proliferation; and that recurrent waves of internal pressure are a contributing factor.

It is worth noting that neither peristaltic-like activity nor its stretching effect on pedicular outgrowth was seen or even imagined before the cinematic record was made. This, by telescoping into 10 minutes that which ordinarily takes from 24 to 48 hours, brought out many features which were not detectable under usual periods of study.

Knowing now that these things do take place opens up opportunity to explore many hitherto unsuspected interdependencies between structural increase and physical force.

While cell increase in number is of course the dominant growth activity in pedicular production, cell specialization and cell segregation are obviously concerned in the form it takes. And since there is increase in mass it is also obvious that constructive substance increase is a continuing participant.

It is well to re-state that the materials for this constructive increase are brought to the growing organism by the hydroplasm. This was first remarked by Brooks (178), and was rediscovered and proven by Hammett and Padis (226) and Hammett and Chapman (214).

Our first observations established that all during pedicular and hydranth development, up to the time the animal feeds itself, the direction of hydroplasmic streaming is out into the growing part, and that many granules of variable size are carried in the stream.

Once the animal becomes capable of feeding itself, however, the current is now away from and now into the gastric cavity.

In the second series of observations chemical tests established: first, that the granules carry free amino acids; second, that these derive from partial digestion of the protein ingested by mature feeding animals; third, that the hydroplasmic reaction for free amino

acids is greatest in the terminal reaches; and fourth, that amino acids concentrate in the distal growing substance of the immature animal.

No more is needed to demonstrate that the materials for constructive substance increase are brought to the growing animal by the hydroplasm.

Once the bud has produced the pedicel it proceeds to produce the hydranth. It first elongates slightly without annulation (Plate 1, Figure 4) and then expands into the cone-like structure pictured in Plate 2, Figures 1, 2, and 3, and Plate 5, Figure 2.

Now the concentration of SH reaction in the bud persists during its elongation, and is still strong in the distal cell layer of the early cone (182). This is chemical evidence that proliferation is actively contributing to growth from bud to cone.

What about cell specialization and cell segregation? Comparing Figure 2 with Figure 1 of Plate 5 it is seen that the *kind* of cells constituting the cone is essentially the same as that of the bud. There is thus no good cytological evidence that any particular shift in cell specialization or Differentiation activity from that originally present has taken place during the expansion. Contributory to this assumption is the fact that the differentiation-forwarding amino acid tyrosine (191:196:214) is only found in weak concentration in bud and cone, while it is found in good amount in the next distinctive structure (Plate 3, Figures 1, 2, and 3) where change in cell character is evident.

Finally, the pictures show that cell segregation within the cone is not particularly different from that of the bud. Both structures are simply constituted of an outer band of cells enclosing a cavity within which is contained the streaming and food-bearing hydroplasm. True it is that the contour has changed; but the arrangement of the cells is essentially the same in both bud and cone. Thus there is no good cytological evidence that any particular shift in cell segregation or organization activity from that originally present has taken place.

Therefore, since there is no positive evidence that either differentiation or organization are particularly active in this phase of hydranth growth; while there is positive evidence from SH concentration and cell increase in number that proliferation is active; it is concluded that proliferation is the dominant growth activity concerned in bud growth to cone. In other words, growth from bud to cone is paced by cell

increase in number, while cell specialization and cell segregation are but runners up, far back in the field.

The fact that constructive substance increase is also a participant in no wise detracts from this conclusion (14).

Just as pedicular elongation is factored by recurrent outward surges of internal pressure brought about by hydroplasmic pulses emanating from peristaltic activity within the hydrocaulus; so is the expansion of the bud into the cone "facilitated" by like activity within the pedicel. It could be that the lessened proliferation activity of the cone relative to the bud (182) favors the expression of this mechanical force.

It is of course impossible to here reproduce the cinematic record wherein this outsurging is clearly and dramatically shown. The best that can be done is to give single cuts from the film. Figures 1 and 3 of Plate 2 show the hydroplasmic stream in its expanded state. Figures 2 and 4 show it as constricted. The influence of these changes on a closed semi-elastic tube is easy to imagine.

A comparison of Figure 1 Plate 3 with Figure 3 of Plate 2 makes evident that the cone stage of hydranth growth is distinct and separable from the bud. This serves then as a clean-cut mark of proliferation expression in hydranth growth.

Once the cone is established the hydranth goes on to develop into a definitive structure approximating a pedunculated truncated cylinder. Characteristic examples of successive stages of the progression are shown in Figure 4 of Plate 2, and Figures 1, 2, and 3 of Plate 3.

The pictures are sufficient demonstration that this change is one of structural differentiation. The animal progresses from a state of virtual homogeneity to a state where the antecedents of the mature structures are visibly in the making. This suggests that cell specialization has taken over from the antecedent dominance of proliferation, and has itself become the dominant growth activity concerned in this stage of hydranth growth.

Evidence establishing this as an allowable conclusion is available from structural, cytological, chemical and *a priori* points of view.

A priori, cell specialization can be taken to be an instigator of structural differentiation (14).

Structurally, the change from cone to cylinder (Plate 2, Figure 3 vs. Figure 4) is not implemented by anywhere near as great an in-

crease in cell mass as is the change from bud to cone (Plate 1, Figure 3 vs. Plate 2, Figure 1). This is *prima facie* evidence of a lessened contribution of proliferation. In other words, proliferation is no major contributor to the change. This is further substantiated by the fact that no particular mass increase occurs as the animal evolves from the state shown in Figure 4, Plate 2 to the state shown in Figure 3, Plate 3.

Cytologically, a comparison of Figure 3 with Figure 2 of Plate 5 shows that the character of the cells constituting the more advanced stage is different from that of the antecedent cone.

Chemically, SH concentration is definitely less in the late cone than in the bud, and markedly less in the cylinder than the cone (182). This is chemical evidence that the contribution of proliferation to hydranth growth decreases as cone changes to cylinder. It suggests that proliferation contribution to cylinder growth is minimal. The chemical findings are interdependent with the structural and there is mutual agreement.

Further,—concentration of the differentiation forwarding amino acid tyrosine is slightly greater in cone than in bud, and markedly greater in cylinder than in cone (214). This supports the idea that the contribution of cell specialization to hydranth growth increases as cone changes to cylinder. And it supports the idea that the contribution of differentiation to cylinder growth is considerable.

The cumulative evidence of these interdependent data leads to no other conclusion but that Differentiation is a major growth activity concerned in the growth progression from the state shown in Plate 2, Figure 3 to that shown in Plate 3, Figure 3. Clearly the contributions of Proliferation and Constructive Substance Increase are relatively minor in degree.

But what about Organization? Undoubtedly its contribution to the process as a whole is here greater than in the growth from bud to cone. The formation of the little circular shelf by which the animal is held attached to the hydrotheca at its jointure with the pedicel is presumptive evidence of cell segregation as a growth expression (Plate 3, Figures 1, 2, and 3: Plate 5, Figure 3). But the disassociation from the hydrotheca of what is to become the gastric cavity, and the appearance of the cylinder, is rather a product of cell specialization—

aided and abetted by shifts in internal hydroplasmic pressure—than of Organization *per se* (14).

Furthermore, if one compares Figure 3 of Plate 5 with Figure 1 of Plate 6, it is immediately evident that the cells of the cylinder have by no means segregated into the definitive structures of the fully-developed feeding animal. It may therefore be assumed that the relative participation of Organization in the picture as a whole is here considerably less than that of Differentiation.

From all this it may then be postulated that since cell specialization sets the pace, it may be taken as the dominant growth activity concerned in the formation of the pedunculated truncated cylinder stage of hydranth growth from the antecedent cone.

The cinematic continuum shows that during the early change from cone to cylinder, constriction (Figure 2) alternates with expansion (Figure 1). In fact, during this phase of growth the hydranth will take the form shown in Figure 2 and then revert to that of Plate 2, Figure 3 many times. Under the conditions existing during the taking of the film, a complete cycle from constriction to expansion and back again to constriction may take place within the short time of 60 seconds. Photographic evidence of this is given in a series of consecutive shots published elsewhere (181). The phenomenon is *not* an expression of alternating differentiation and "dedifferentiation." It is due to a combination of assumption of free rhythmicity by the differentiating cells of the presumptive gastric cavity and the surges and recessions of hydroplasmic pressure originating in peristalsis-like activity within the pedicel.

As the cells progress in specialization, however, the reversion to the expanded state is less and less complete, and the future gastric cavity becomes and stays entirely disassociated from the hydrotheca as shown in Figure 3, Plate 3. Comparison of Figure 3 with Figure 4 is pictorial representation of the fact that these changes do not stop here. They continue, and are continuing factors of importance to the growth and activity of the animal.

The fact that the cone shows no free rhythmicity while such is present in the cylinder is pretty good evidence that the change from cone to cylinder involves a specialization of the cells, and hence that the change is expression of differentiation progression.

How pressure changes contribute to the attainment of hydranth

form is unknown. That they do contribute can be little doubted. And while the phenomenon is another example of the rôle of physical force in growth and form as postulated by d'Arcy Thompson (262) it must not be forgotten that what the form is to be is determined by genetic direction and not by internal pressure. Which is to say, the expression and extent of pressure effect is limited by genetic determination.

Conversely it is questionable that genetic determination *per se* is limited by internal pressure changes. But it is quite possible its expression and the extent thereof may so be. It's a pretty point; light on which might possibly be obtained by experiments with immature hydranths isolated from the colonial hydrocaulus. The question really is: Is there interdependence here between direction and state of substance, or is the relation a one-way street? The problem is being looked into by Dr. Theodore Hauschka of The Institute.

Aside from this, it is probable that alternate compression and decompression of the presumptive gastric cavity by changes in hydroplasmic pressure is an adjuvant to its disassociation from the chitinous hydrotheca as its cells progress to specialization.

A comparison of Figures 1, 2, and 3 of Plate 3, with Figures 1, 2, and 3 of Plate 2, makes evident that the cylinder stage of hydranth development is distinct and separable from that of the cone. This then may serve as a clean-cut mark of differentiation expression in hydranth growth.

From the cylinder stage the animal proceeds directly into the complete stage, and its growth is essentially over. It passes from the state pictured in Figure 3, Plate 3, through that of Figure 4, to that of Figure 1, Plate 4. The details of the end-result are best shown in Figure 1 of Plate 6.

The growth progression results in the complete disassociation of the hydranth from its hydrotheca, save at the basal shelf and its jointure with the pedicel; and the formation of tentacles, mouth, oesophagus, and gastric cavity. The inchoate cell mass of the cylinder segregates into the definite functionable structures of the completed animal.

Clearly the basic growth activity of Organization is a major contributor to this—the ultimate phase of hydranth growth.

The fact that SH concentration is weak and discontinuous in the distal tissues of the late cylinder (182), and the fact that the progression seems unaccompanied by any particular increase in mass, allows

the conclusion that both Proliferation and Constructive Substance Increase contribute but little to the final stage.

While cell specialization is undoubtedly a continuing participant in the beginning progression from cylinder to completed animal, it is equally certain that the difference between the two is essentially a difference of organization. Hence it is allowable to conclude that Organization is the dominant growth activity concerned here.

The progress of the change is accompanied, as are all the preceding changes, by strong outward surges of hydroplasmic pressure. And as these are there taken as adjuvants to growth, so may they be similarly taken here.

Now all through its growth the hydranth produces an enclosing chitinous envelope. Up to the cone stage the two are firmly affixed. With the onset of differentiation dominance there is separation. This increases as the cylinder stage becomes established (Plate 2, Figure 4; Plate 5, Figure 2 vs. Plate 3, Figures 1, 2, and 3; and Plate 5, Figure 3). It is, however, confined to the future gastric cavity. It does not extend to the distal cell mass. As organization sets in, however, the latter begins to be separated (Plate 3, Figure 4). Finally the animal comes to be free within the hydrothecal envelope save for its basal jointure.

But the distal chitinous cover is still present. Obviously the animal cannot emerge from confinement until this covering sheet is disposed of. The cinematic record suggests that disposal is factored by pressure from within. The film shows the completely organized hydranth repeatedly surging against the cover with great force. Not only is this stretched outwards; but even the body of the hydrotheca is stretched lengthwise, so great is the pressure exerted. Here as elsewhere it is peristaltic-like activity which is responsible for the pressure changes.

The implication of changing hydrothecal shape in Obelia speciation has been previously noted.

While mechanical force may be a culminating factor in the actual removal of the distal disc, the potentiality for removal may be otherwise subtended.

Since the normal hydrotheca of the functioning animal has no ragged edge of fragments of the distal cover (Plate 6, Figure 1) it may be concluded that the circumferential jointure between the two

is the line of partition. This suggests the chitin of the jointure region differs from that of the rest. Clearly the potentiality for partition is underwritten by the production of this difference.

It would be interesting to know whether the difference is the result of a localized difference in amount of chitin deposition; a localized difference in chitin composition or polymerization; or whether it is a thinning out after deposition through localized action of enzymes peculiar to the marginal distal cells and peculiar to the stage of development. Perhaps all these and even other factors contribute to the preparation for removal. And indeed it is possible that not only the jointure, but also the entire cap is affected.

A comparison of Figure 3 of Plate 3 with Figure 1 of Plate 4; and of Figure 3 of Plate 5 with Figure 1 of Plate 6 makes evident that the complete stage of hydranth development is distinct and separable from that of the cylinder. This then serves as a clean-cut mark of organization expression in hydranth growth.

This seems as good a place as any to pause and refresh ourselves with the broad significance of these data.

1. *They show that the growth progression of Obelia hydranths is marked by a succession of definitive structural forms, the attainment of each of which is dominated by a single basic growth activity. And they demonstrate that Obelia exhibits ALL the basic growth activities in full expression during the period of observation in such fashion that the expression of each is distinct and separable from that of the others.* Which is what we set out to do.

2. *They suggest that the growth progression from one structural stage to the next may be "facilitated" by internal pressure changes.*

3. *The fact that shifts in hydroplasmic pressure cause outward expansion and recession of the hydranth throughout its growth and the possibility that the expression of each growth activity is characteristically affected thereby, suggest that growth intensity is rhythmical rather than constant.*

4. *The fact that one structural state melds into the next shows that growth is a continuum and not a series of saltations.*

5. *They show that growth is not single but a compound of separate though interdependent activities.*

6. *They show that the participation of each activity in organism production is not a constant but a continuously changing value.*

7. *And they show that even though single growth activities may dominate the attainment of definitive stages, others than the dominant contribute to the progression.*

Visualization of the principles stated in the last four sentences is made in Chart 1. This shows the assumed relative participation of Proliferation, Differentiation, and Organization in the attainment of each of four Stations along the growth progression from hydranth bud to the completed animal. The estimate was derived from the correlated chemical, cytological, and size data outlined in preceding paragraphs. It is, however, only a guess. Nonetheless, it is believed to be a valid approximation. The charting was restricted to the noted activities because these dominate the progression; because the principles are adequately and simply illustrated thereby; and because data for estimating the contributions of other participants on the same basis were not available.

Station 1 on the chart represents the assumed relative participation of proliferation, differentiation, and Organization in the progression from the ball-like bud to the elongated state (Plate 1, Figure 3 to Figure 4). Station 2 represents the guess for the change from the elongated bud to the large cone (Plate 1, Figure 4 to Figure 3, Plate 2). Station 3 is the estimate placed on the relative activity incident to the production of the late cylinder from the cone (Plate 2, Figure 3 to Figure 3, Plate 3). And Station 4 represents the alleged contribution of the three activities to the final growth change (Figure 3, Plate 3 to Figure 1, Plate 4: better Figure 3, Plate 5 to Figure 1, Plate 6).

From the pictures and chart it is plain that growth in Obelia is something other than increase in weight or size. Growth certainly is not over with the attainment of the structure pictured in Figure 3, Plate 5. For the animal goes on from there to acquire tentacles, a mouth, oesophagus, and stomach. Yet this progression does not involve any appreciable increase in substance or dimension. What it does involve in dominant measure is nothing more nor less than segregation of specializing and specialized cells into definitive structures whereby the hydranth becomes a functionable organism. Without this there would be no functionable organism. To claim that this organization is not growth, is to claim that in Obelia at least all growth stops before a functionable animal is produced. Which is, of

course, ridiculous. The same principle holds true with respect to Initiation, Proliferation, and Differentiation.

The demonstration that growth in Obelia is not simply substance increase, but this plus other basic activities, allows conclusion the same is true for all other growing things, since all exhibit all these activities at one time or another.

Such being so, any formulation of growth which aims at comprehensiveness must include not only constructive substance increase but also the four other basic and interdependent variables, viz.: Initiation, Proliferation, Differentiation, and Organization. To these should be added the non-growth variable Maintenance, and the non-determinative physical adjuncts when indicated.

In view of this it may be well to consider whether Time is the best frame of reference for formulation. Time as duration is of course the most convenient marker of progress from lesser to greater weight; from fewer to larger numbers; and even from less to more specialization.

But when we consider growth from the point of view of its function, which is the production of a functionable organism or structure; we see that growth in the abstract is not a matter of the time required for exercise of this function, but of the sequence of processes and structural changes whereby this function is accomplished. In other words the mark of growth is sequence rather than duration.

Since then the functionable organism or structure is attained by the sequence or progression from one definitive structural state to the next; the which is instigated and determined, not by Time, but by the exercise of the basic growth activities of Initiation, Proliferation, Differentiation, and Organization, it would seem as if structural age is the better frame of reference for comprehensive formulation than time.

Agreeable to this conclusion is the fact that sequence is genetically determined and essentially independent of environment, while duration is subject to alteration by many environmental influences of which temperature is but an example.

Finally, the substitution of sequence for duration is in line with the well-known fact that physiological age is a better standard for evaluation of performance than chronological.

This does not mean that time as duration is never to be intro-

duced into the formulation. It merely means that it is better not used as a frame of reference, and that its introduction is unnecessary when growth is considered in the abstract. Its use is of course indicated when interest is in duration, and the influence of any factor thereon. But even here structural age is the more valid primary basis, since time is undeterminative of the event.

What we want the formulae to show, then, is not the time required to produce an increase in any aspect of growth, or the time required to progress from one developmental state to the next; but the extent to which each of the basic growth activities and adjuncts contributes to such progression.

That is to say, *the formulae should picture the intensity and correlation of Initiation, Proliferation, Differentiation, Organization, Constructive Substance Increase, and Maintenance at any and all stages of the growth progression from its inception to its completion.*

This is a problem for the mathematical experts. It is herewith placed squarely in their laps. In so doing I would emphasize:

1. The advisability of abstract formulation. I.e., do for growth what Willard Gibbs did for thermodynamics.
2. The possibility that the Woodgerian System is a better tool for formulation than the mathematics of engineering as at present constituted.
3. The possibility that since all the basic growth activities are interdependent, there is no master reaction; no independent variable.
4. The probability that molecular turn-over is a suitable common denominator, since it is a phenomenon common to all the basic growth activities.

Let us return to our hydranth.

When we left it, it had completed its growth, emerged from its hydrotheca, and become a feeding animal.

The older hydranths are slightly less translucent and tender-seeming than the newly emerged. This may be due either to the entropy of aging or to some short-lived and slight increase in substance and selective assimilation; or to both. At any rate the change is of little importance to the present thesis.

The animal exists full-blown and functionable for a variable but undetermined number of days. Its cells are concerned, not with

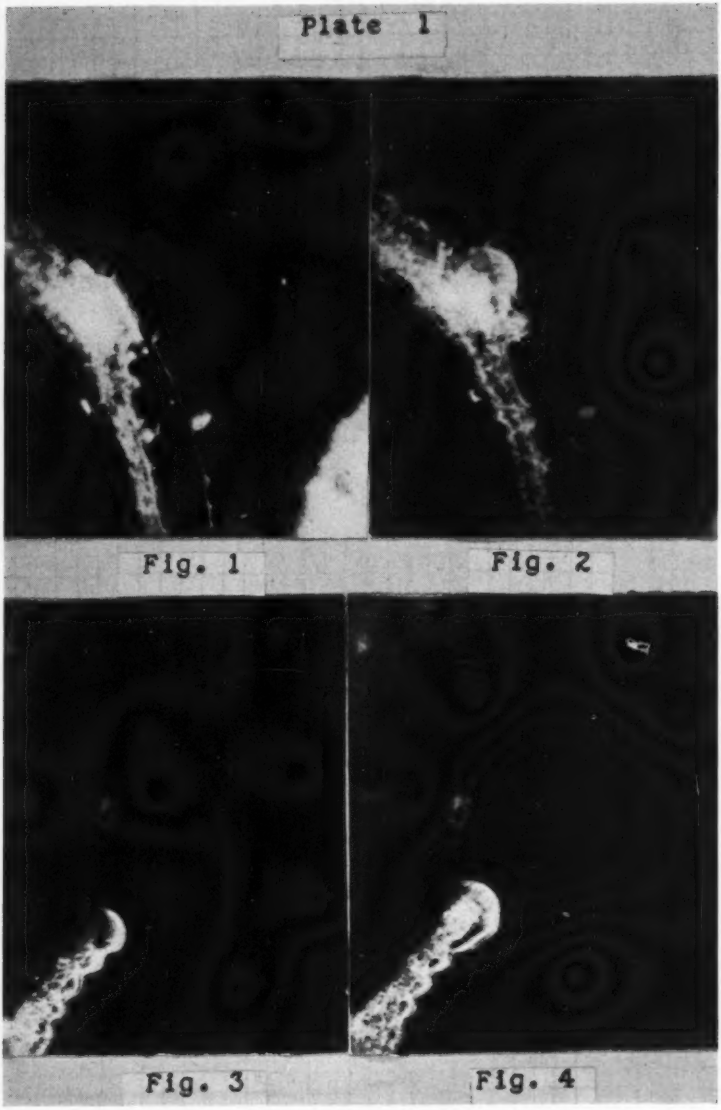

PLATE 1

Figure 1. Anlage of an Obelia Hydranth.
Figure 2. Hydrocaulal Bud of an Obelia Hydranth.
Figure 3. Pediculated Bud of an Obelia Hydranth. Early Stage.
Figure 4. Obelia Hydranth Bud. Late Stage.

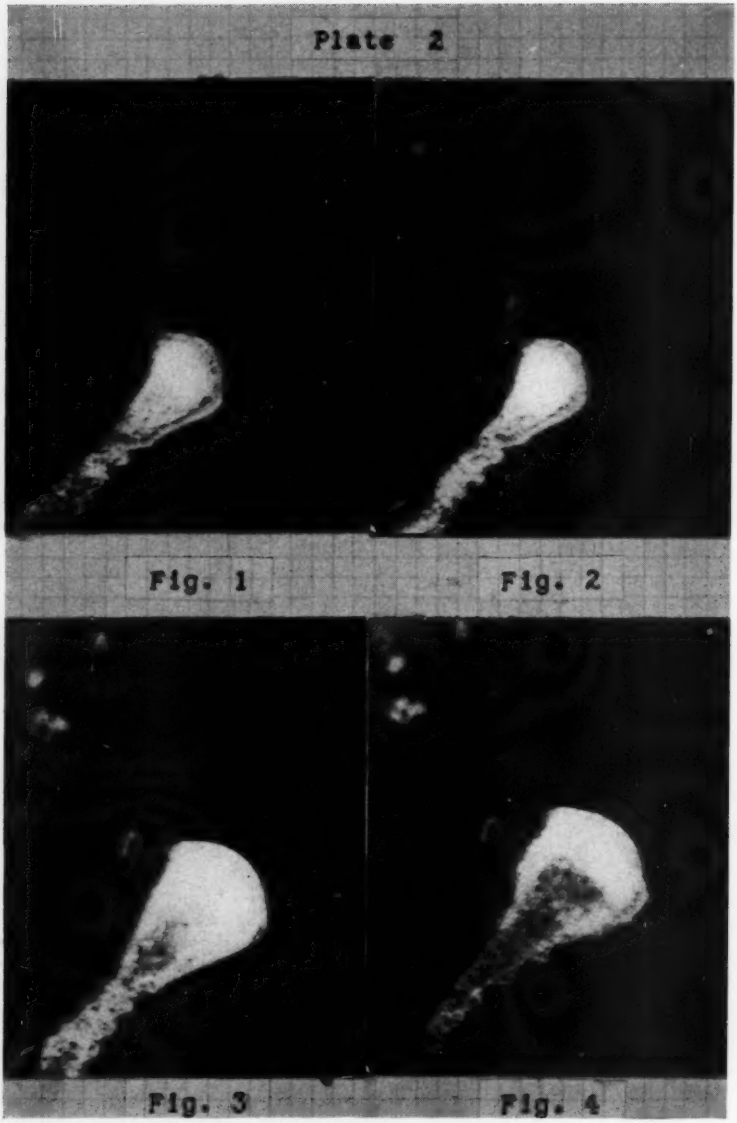

PLATE 2

Figure 1. Cone Stage of an Obelia Hydranth. Expanded Hydroplasm in Pedicel.
Figure 2. Cone Stage of an Obelia Hydranth. Contracting Hydroplasm in Pedicel.
Figure 3. Late Cone Stage of an Obelia Hydranth.
Figure 4. Beginning Cylinder Stage of an Obelia Hydranth. Constricted Hydroplasm in Pedicel.

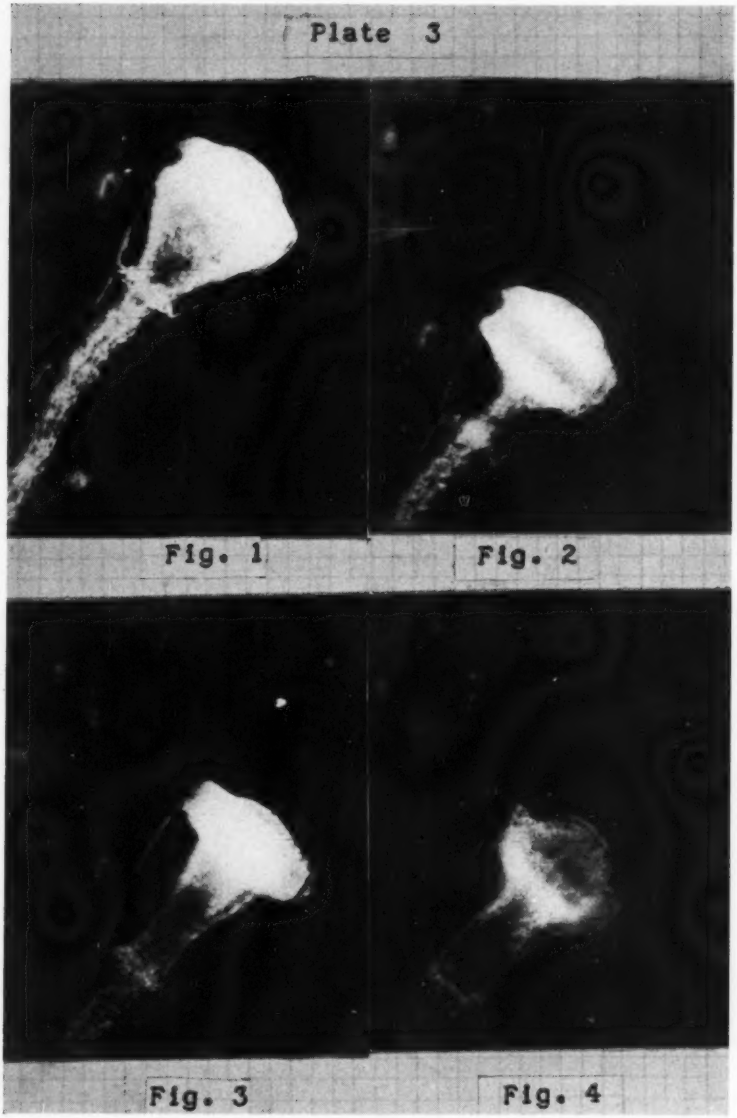

PLATE 3

Figure 1. Cylinder Stage of an Obelia Hydranth. Presumptive Gastric Cavity expanded.
Figure 2. Cylinder Stage of an Obelia Hydranth. Presumptive Gastric Cavity constricted.
Figure 3. Late Cylinder Stage of an Obelia Hydranth.
Figure 4. Early Complete Stage of an Obelia Hydranth.

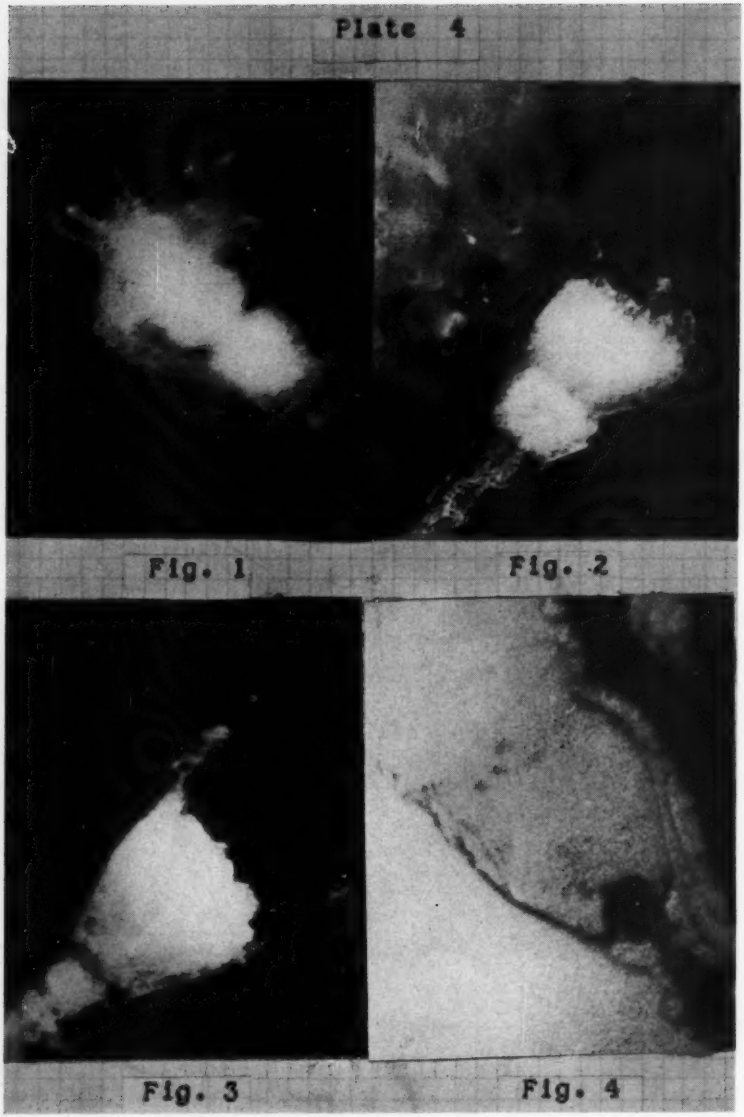

PLATE 4

Figure 1. Complete Obelia Hydranth.
Figure 2. Beginning Regression of an Obelia Hydranth.
Figure 3. Senile Obelia Hydranth.
Figure 4. Empty Hydrotheca of an Obelia Hydranth.

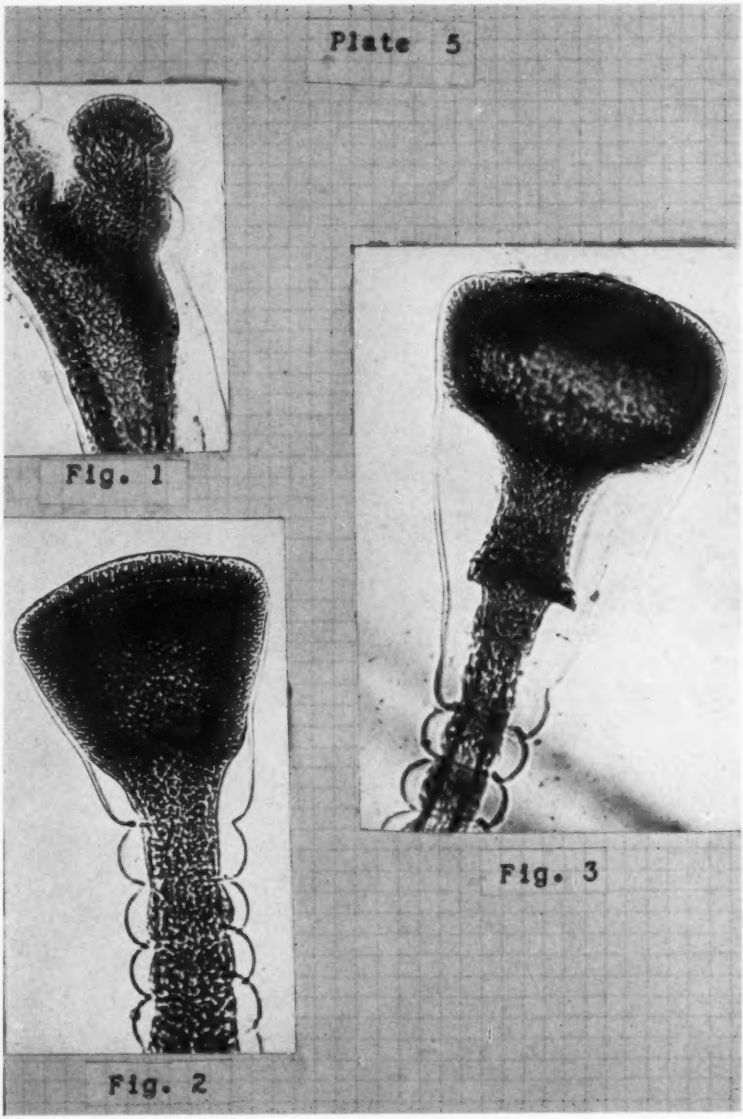

PLATE 5

Figure 1. Beginning Bud of an Obelia Hydranth.
Figure 2. Cone Stage of an Obelia Hydranth.
Figure 3. Cylinder Stage of an Obelia Hydranth.

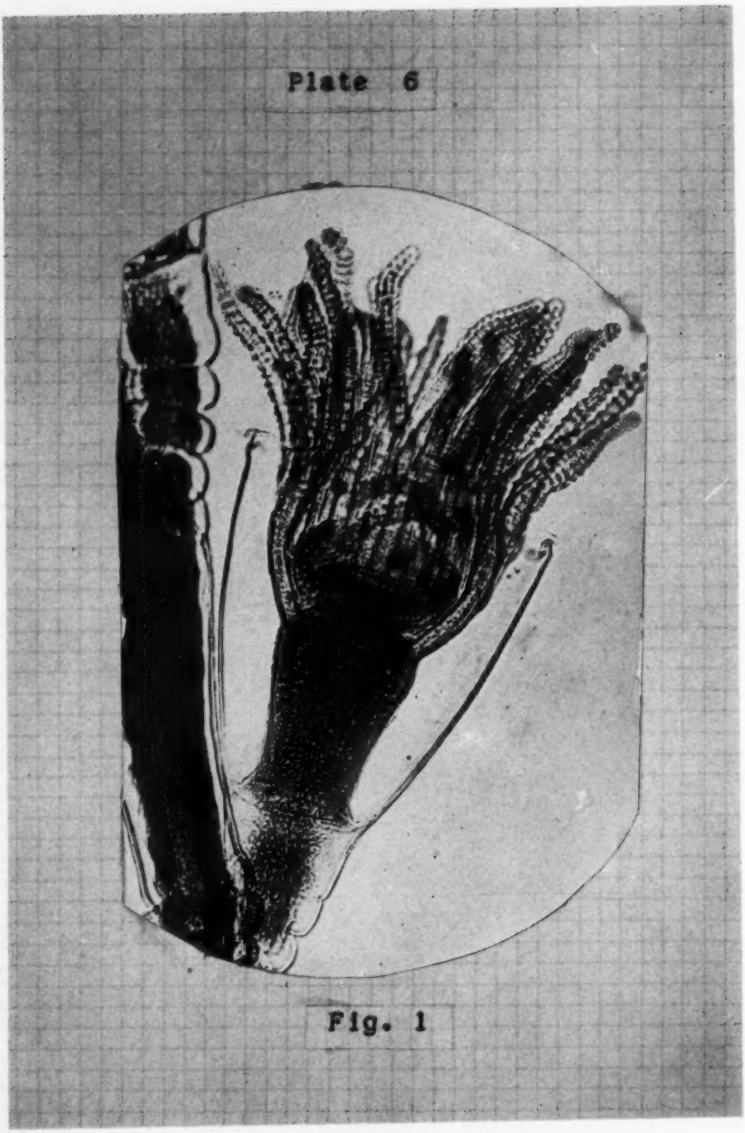

PLATE 6
Figure 1. Complete Obelia Hydranth.

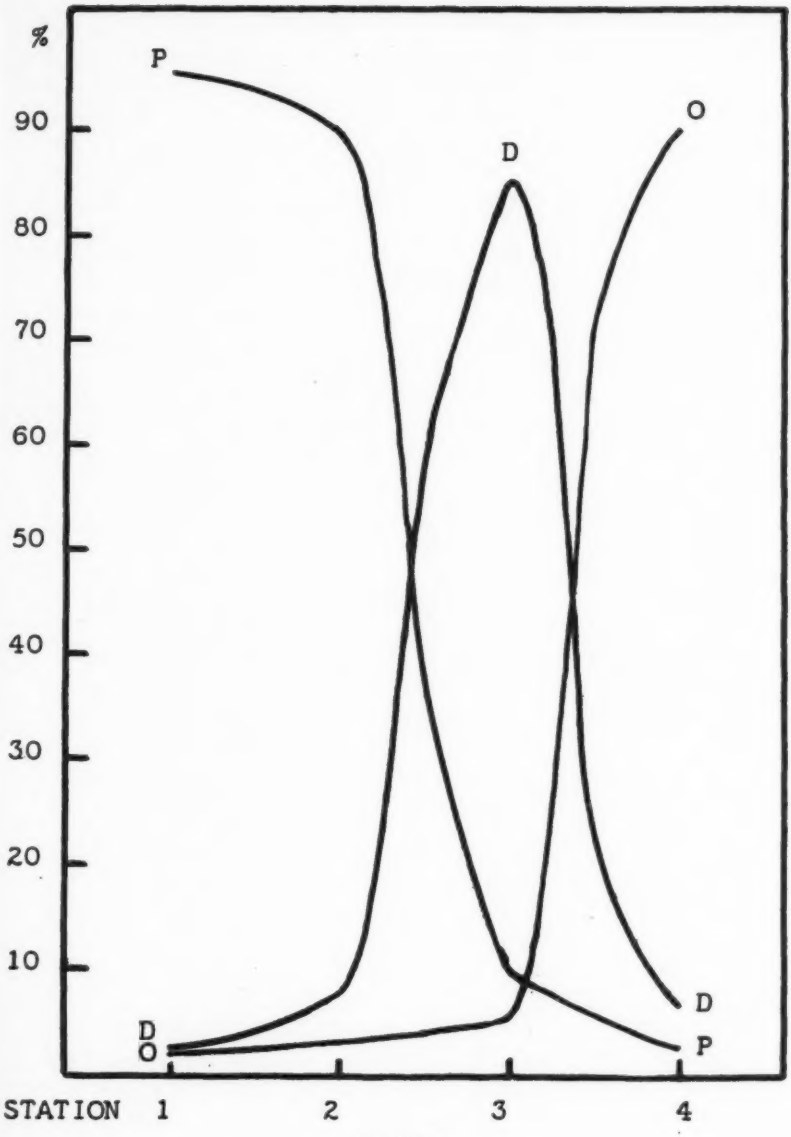

CHART 1

An Estimate of the Extent to which Proliferation (*P*), Differentiation (*D*), and Organization (*O*), Contribute to Successive Structural States Exhibited by an Obelia Hydranth during its Growth.

growth, but with the business of living. This then is a state in which Maintenance is the dominant activity (14).

A shift in direction of hydroplasmic streaming is integrated with the shift from growth to maintenance. As previously noted, all during growth the current is out into the developing part, carrying to it the materials for substance increase. Once the hydranth completes its growth, however, and becomes a feeding animal, the current is as often away therefrom as towards. Thus the ingested food is carried away and distributed to the rest of the colony for use in growth and maintenance (214:226).

Sooner or later the animal begins zugrunde gehen. The tentacles are withdrawn into the hydrotheca (Figure 2, Plate 4); the mouth and stomach lose their identity as they shrink into an amorphous cellular mass (Figure 3, Plate 4); and the hydranth dissolves away leaving an empty hydrotheca and a useless remnant which is soon cast aside (Figure 4, Plate 4). Thus the life-cycle of an Obelia hydranth is completed.

Since the feeding animal is structurally separable and distinct from the growing animal on the one side (Figure 1, Plate 4 vs. Figure 3, Plate 3), and from the disintegrating animal on the other (Figure 1 vs. Figure 3 of Plate 4) its persistence in the complete state is clearly a clean-cut mark of Maintenance activity expression.

The last phase of hydranth existence is analogous to the senile dissolution exhibited by most aging organisms. It is endogenously determined, and it is a phase in which catabolism is the dominant activity. Thus—

1. Colonies as collected or in culture may present hydranths in all intermediate states of dissolution from the stage pictured in Figure 3 to that shown in Figure 4 of Plate 4.

2. The direction of hydroplasmic streaming is predominantly away from such senile animals in contrast to being out into those that are growing.

3. The cinematic continuum of a single regressing hydranth shows this steadily decreasing in size; shows the hydroplasm streaming away therefrom; shows a central thinning out of the disintegrating cellular mass; shows the particles freed thereby flowing down into the hydroplasm; and shows them being carried away in the down-streaming current.

4. Chemical tests for free amino acids in senile hydranths found in colonies as collected shows such to be concentrated within the disintegrating cell mass and not upon its surface (214).

The fact that amino acids are liberated in the dissolution; the fact that these are carried away and presumably distributed to the rest of the colony by the hydroplasm; and the fact that the growing animal uses free amino acids for its substance increase; suggest that those liberated in the catabolism of the senile structure may be re-utilized in the growth of other parts.

Furthermore, the fact that solid particles are carried away from the *disintegrating hydranth* by the hydroplasm, combined with the fact this flows out into as well as away from the *functioning animal*, suggests that indigestible residues are thus disposed of by excretion. In other words, excretion as well as ingestion is a function of the complete hydranth.

5. Other evidence that regression is catabolic is found in a record of the electrical correlates of Obelia growth and dissolution (181). This shows "—that the voltage gradient rises in a characteristic fashion during the course of developmental growth from the anlagen to the complete functional stage. When function ceases the voltage gradient drops rapidly, then more slowly as regression continues, until suddenly, as senile dissolution takes over, the polarity of the gradient is reversed—."

Now voltage gradient is here a product of underlying chemical reactions. And reversal of gradient direction is indicative of reversal in direction and nature of these reactions. In other words, the voltage reversal is evidence that the constructive reactions of anabolism have shifted to the destructive reactions of catabolism.

Thus physical, chemical, cytological, structural, and physiological data, all give proof that regression is a usual terminal phase of hydranth existence; that this is endogenous in origin; and that its sequence is catabolism.

Comparison of Figure 3 with Figure 1 of Plate 4 shows that the senile organism is separate and distinct from the functioning. Hence we have in this a mark of regression from Maintenance and a mark of assumption of catabolic activity.

To summarize: The Obelia hydranth presents a succession of separable and distinguishable structural states, the attainment of each of

which is dominated by a single basic activity, viz., in the order of appearance, Initiation, Proliferation, Differentiation, Organization, Maintenance, Regression, and Catabolism. The percentage of organisms going from one state to the next is clearly a measure of the extent to which the dominating activity is expressed; and the overall progression during growth is a reasonably accurate mark of Constructive Substance Increase. Thus the suitability of Obelia as test material for evaluation of the specific rôle in growth of each of the naturally occurring tissue components of general distribution is demonstrated.

Obelia Growth: Recurrent Growth

Once the hydranth is dissolved away and its substance returned to the colony, a new organism may emerge from the distal annulation of the pedicel and follow the course described in preceding paragraphs.

This phenomenon is called Recurrent Growth because it is production of a hydranth from a site where an animal had previously emerged, grown, lived, and died, as distinct from the production of a hydranth from a site where none had previously existed.

Sometimes the bud starts while the hydrotheca of the preceding animal is still attached to the pedicel. Usually the old shell is cast off either before or shortly after the new budding. But sometimes this does not occur. In such rare cases we see a new completed animal at the distal end of its own pedicel extending beyond the hydrotheca of the old.

In any case these recurrent hydranths exhibit all the basic growth activities and are thus integratible into the growth picture of the colony as a whole.

While the trend of influence of any compound on developmental growth is set by the hydranths; other growth expressions obtain in the colony which are useful contributors to interpretation.

Obelia Growth: Stolons

Colonies in culture, more often than not, produce stolons from the cut end of the hydrocaulus. Since the stolon is part of what may be considered a vascular system, its production is a type of Vasculogenesis.

It is a nice point whether the phenomenon is to be considered a

regeneration of the cut away hydrocaulus. Yet stolon production must here involve reconstitution of hydrocaulal coenosarc cells—since no stolon tissue is conceivably present at the level of cutting—and reconstitution is a characterizing pre-requisite of regeneration (183: 184:197). If it is regeneration it is of the 3rd or 4th degree, not of the 1st.

Regardless of this, stolon production is here an expression of initiation activity. Stolon growth in length is a matter of cell increase in number with its concomitant endowment of constructive substance increase. Since chemical tests show SH to be in greatest concentration at the tip (182), the inference is that this is the region of most active proliferation. Apparently the stolon grows outward in somewhat the same fashion as a root.

Occasionally two stolons are produced Y-shape from the cut end of the hydrocaulus. The inciting agents for such doubling have not been determined.

Sometimes, but not often, a hydranth is produced at the distal end of the growing stolon. It is not a branching off, but a shift of the distal region from stolon to hydranth production. One or two mild annulations appear, and then a bud, which may grow through the usual definitive structural states into a complete feeding animal.

Cytologically and structurally the shift is a specialization of stolon cells. Chemical tests show accumulation of the differentiation-forwarding amino acid tyrosine at the site of the change (214). The conclusion is that the shift is an expression of differentiation activity. Since it involves a change in cellular production direction it is conveniently designated as Metaplasia.

The direction of hydroplasmic streaming is predominantly out into the growing part (226). And since the hydroplasm is continuous with that of the colony it may be presumed that internal pressure changes contribute to stolon out-growth as they do to hydranth.

In this connection Billard (172:175:176) has reported that stolonization is influenced by water currents acting externally. In a somewhat different category are the experiments of Hammett and Rivard (232). Here centrifugal force was applied to colonies before stolons appeared and the effect thereof on subsequent stolon growth noted. It was found that while the outward pull did not affect stolon production, since stolons emerged from as many uncentrifuged hydro-

cauli as from the centrifuged, the subsequent growth in length was less in the centrifuged than in the uncentrifuged, and the retardation was greater, the greater the centrifugal force applied. Furthmermore, more metaplasic hydranths were produced by the tests.

Now these reactions were not the result of mechanized force applied during growth as is the case with hydroplasmic pressure internally and water currents externally. They were instead the consequence of the influence of mechanical force on the balance of relations preceding growth.

The different results suggest that different processes were affected in the two sets of data by the same general type of physical agent, i.e., mechanical pressure.

It may be assumed that hydroplasmic pressure and possibly water current pressure act largely as stretching factors. And just as stirring of mixtures in a beaker facilitates their reaction, so is it possible that stretching may in some cases facilitate the chemical reactions of growth and its consolidation.

It is otherwise with pressure applied prior to stolon emergence. Here no stretching of stolon tissue can occur since none is present for such reaction. In the absence of direct evidence the best guess is that the centrifugal pull affects an over-the-usual concentration of chemical differentiators at the cut end of the hydrocaulus. The balance of growth determinants is certainly upset, and since there is less growth in length and more metaplasia, the inference is that the change is in the direction of relative increase in cell specialization factors. That such translocation of growth determinants may be effected in Obelia by gravity and electrical current has already been established by Davenport (185) and Lund (244-249).

Now it is to be noted that pressure does not alter the sequence or nature of growth; but merely its extent and duration. In other words the effect produced by physical force is determined by the state of the reaction basis at the time pressure is applied. The moral of all this is that physical force is not primary in the determination of growth and the forms it takes, but secondary to chemical activity as set by genetic direction (212).

To summarize: Stolon production and growth by Obelia colonies in culture is effected by the basic growth activities of Initiation, Proliferation, and Constructive Substance Increase, to which Differentia-

tion is added when Metaplasia supervenes. With the exception of substance increase the expression of each activity is separable and distinct from that of the others. Stolon growth is thus an adjunct in determining the reaction of basic growth activities to experimental procedures.

Obelia Growth: Gonangia

Gonangial growth is twofold, like that of the animal. There is growth of the gonophores (hydranth) and growth of the enveloping gonotheca (hydrotheca). These undergo a succession of growth changes from which definitive structural states may be picked as was done in the case of the hydranth.

While it would be useful to have a cinematic record of gonangial growth, such has not been made; nor are photographs available of the definitive states. Photographs have not been taken because interest is primarily in the hydranth and only adventitiously in the gonangium. This comes first from the fact that colonies in culture always have hydranths, while many have no gonangia; second from the fact that an obelia colony is a colony of hydranths and not a colony of gonangia; third from the fact that hydranth growth does not depend on the gonangia, while the gonangia do depend upon material supplied by the feeding hydranths; fourth from the fact that each growth activity is expressed in clean-cut fashion in the hydranth, while such is not the case in the gonangium; and fifth and finally because gonangial growth is not completed within the usual 24-hour period of observation, or even long thereafter. Because of all this, verbal exposition plus reference to diagrams given in an earlier report (119) is sufficient for the present.

The gonangium buds out from the hydrocaulus quite the same as does the hydranth. In *Obelia geniculata* this occurs close to a basally situated hydranth-bearing pedicel. Although gonangial anlage have not been noted, because not looked for, the emergence of a bud presupposes the antecedent existence of such an anlage, and thus may be taken as a mark of initiation activity expression.

From the bud there is produced by lateral and longitudinal growth an annulated inverted cone, the distal end of which is sealed by a thickish disc, and within which the future medusae emerge from indistinguishability into recognition. Since this is the sequence of greatest absolute and relative increase in substance and cell number, it is

designated as the period of proliferation dominance. And the attainment of the above-described structure is taken as a mark of proliferation activity expression.

There is no question but that processes specific to cell specialization and cell segregation are considerable contributors to this progression, and to greater extent than is the case in the comparable sequence in the hydranth. Nonetheless, despite the fact the relative participation of cell increase in number is less in degree here than in the hydranth, justification is had for assigning proliferation as the dominant growth activity, since it is during this change that medusal size growth is considerable.

Up to here the gonotheca is full, and even crowded with the enlarging medusae. But from here on, marked changes occur. The gonotheca elongates; its annulations are smoothed out; it bulges taperingly to narrow at its distal end; and the capping disc becomes a cylindrical plug. It is no longer filled by the medusae. These are now strung along the blastostyle which is stretched from plug to base. And there is clear space all around.

The medusae also undergo change. They become more clean-cut and individual. Their translucency increases. Because of these changes the sequence is designated as a period of differentiation dominance. And the progression from the antecedent cone to the above-described structural state is taken as a mark of differentiation activity expression.

Again there is no question but that cell specialization has set in prior to this change, and that cell segregation is a contributor thereto. But there is absolute decline in cell increase in number and substance from that taking place in the preceding change. Combining this with the clear indication of cell specialization progression the assumption is justified, that relatively, at least, cell specialization is a greater contributor to the sequence than any of the other basic growth activities. It is a matter of the relative *contribution* to the "growth-as-a-whole" during any given phase: it is not a matter of the relative *intensity* of a single expression at different phases.

In the next and final phase of gonangial growth the medusae are liberated aborally one-by-one from the blastostyle and become free-swimming functionable organisms; the terminal plug becomes disattached from the gonotheca; and through the opening thus afforded

the animals emerge into the outside world to meet whatever fate is in store for them.

The open gonotheca is thus a structural state which is distinct from the antecedent where no direct communication with the external environment is had, and where no medusae are free-swimming. The attainment of free-swimmingness and the opening to the exterior are clearly the culminating phases of gonangial growth. As such they are taken as a mark of organization activity expression.

As is always the case, every observation opens up new problems and raises new questions. The timing, exhibited here as well as in the hydranth, between completion of the living organism and weakening of its confining envelope, so that emergence into functioning may coincide with attainment of functionability is exciting of admiration and wonder. It would be mightily satisfying to know what processes subtend the disattachment of the plug and cap; their origin; and what sets them into action at just the right time.

Another probably fantastic idea comes from the attempt to resolve the confusion in Obelia speciation by separating Obelia from Campanularia on the basis of gonophore development within the gonotheca. If this goes no further than medusae, then the animal is said to be an Obelia; but if it goes as far as planulae, then the organism is said to be a Campanularian.

This may be a valid difference. But, on the other hand, planula production is also claimed for Obelia in its metamorphosis from medusa to hydroid (260:261). Granting this, it might be possible for such to occur within the gonotheca under certain conditions, and thus planulae and not medusae would be seen emerging at maturity. And conversely, it might happen that developmental progression in what is called a Campanularian gonotheca, might go on further than the medusa stage under certain conditions, and thus medusae and not planulae would be seen emerging at maturity. A little speeding up of gonothecal chitin polymerization on the one hand, and a little retardation on the other might have such results. The matter deserves experimental attention.

It might be noted that expression of the basic growth and adjunctival activities is the same in principle, whether the hydroid be called Obelia or Campanularia, whether medusae or planulae emerge from the gonothecae. Because of this, either is suitable for our kind of

growth study. But until resolution of identity is clarified it is obviously advisable to use but one form for evaluation of any given compound.

To summarize: Gonangial growth, like that of the hydranth, presents a succession of definitive structural states each of which is separable and distinct from all the others. Bud production is a clean-cut mark of initiation expression; and medusae liberation and free-swimmingness is a clean-cut mark of organization. But proliferation has to be assumed from size increase of medusae with concomitant expansion of the gonangium as a whole; while the indices of differentiation are restricted to gonothecal change in shape, and the change in distribution of the within-contained medusae.

Because of this indecisiveness, and for reasons noted in an earlier paragraph, the gonangium by itself is not the best indicator for conclusion regarding growth activity expression. None the less its growth reactions to amino acids, and the like, are useful supplements to those of the hydranths as shown in previous reports.

Before going on to practical considerations it is in order to review certain general aspects of Obelia hydranth growth derived from observation and experience.

Jacques Loeb (243) long ago reported that growth of Obelia as well as other marine organisms is forwarded by hydroxyl. We have found that increase in sea water alkalinity to pH$\pm$9.4 enhances expression of cell specialization, diminishes that of cell segregation, and has no effect on cell increase in number or the preparatory processes of initiation (231). Evidently changes in sea water pH can have differential effects on growth activity expressions. This means that each activity has its own pH optimum. Thus it is experimental evidence sustaining the postulate that each growth activity has its own set of chemical determinants, participants, and reactions. The broad implication is that the growth of Obelia in its natural habitat is factored by naturally occurring changes in sea water pH brought about by such variables as temperature, sunlight, rainfall, and plankton.

One of the most promising approaches to understanding of the interdependencies of growth is the use and interpretation of electrical correlates as reported by H. S. Burr and his associates (179:180). As already noted, the potential difference between base and distal tip

of the growing Obelia hydranth increases as the growth of the animal progresses from anlage to completion; drops off with cessation of maintenance; and is reversed in direction as catabolism takes over (181).

Furthermore the onset of structural fluctuations during the differentiation sequence is paralleled by the onset of voltage fluctuations, both of which are in marked contrast to the preceding steady states exhibited in the proliferation sequence. Interestingly enough similar voltage fluctuations have been found to occur as cell specialization takes over from cell increase in number in the developing salamander (180) and growing roots of corn (179). This picture is further evidence, if any is needed, that change from hydranth cone to cylinder is expression of differentiation dominance.

This all adds up,—first to the fact that electrical correlates are valid indices of shift in nature of growth activity; second that growth is not single but a compound of activities; and third that each activity is subtended by its own set of chemical reactions, since the voltage gradient is a product thereof. The electrical correlates thus provide one more piece of evidence in support of my concept of growth.

It has already been noted that mechanical pressure emanating from peristaltic-like activity within the hydrocaulus and pedicel is probably a considerable adjuvant to length growth and expansion of the developing hydranth. Further pressure contribution is also had from the peristaltic activity of the developing and developed stomach. The former may well forward the change in cellular distribution as cone evolves into cylinder; and even the separation of the segregated cells into the discrete tentacles, mouth, and oesophagus of the completely organized animal. And internal pressure changes evoked by contraction and expansion of the fully developed stomach undoubtedly aid in the break-through of the animal to the outer world. An analysis of this pressure factor in hydranth growth and emergence in greater detail is being made by Dr. Theodore Hauschka of The Institute Staff, and will be the subject of a later report.

The foregoing deals with the effects produced by pressure exerted *during* growth. A different aspect is presented by the effects produced by pressure (centrifugal) exerted *prior* to growth.

Centrifugal push increases the number of hydranths produced from distal anlagen, and the number regenerating from cut distal ends

of colonial hydrocauli. The subsequent growth of organisms so produced is also increased. The oppositely acting or centripetal pull has an opposite effect, viz., there are fewer hydranths produced and their subsequent growth is less (232). The best present explanation is that growth-forwarding substances immigrate into the region of new hydranth emergence under the influence of positive pressure; and emigrate therefrom under the influence of negative. Much circumstantial evidence from many diverse sources makes this a most attractive hypothesis. None the less before it can become anything more than an hypothesis a great deal of chemical and biological experimental work must be done.

Practical Considerations

Now the purpose of all this is to provide a foundation from which measurements may be made of the extent to which each of the separate basic growth activities is expressed under controlled experimentation. Exposition of the principles of procedure is therefore in order.

Any collection of Obelia colonies is composed of a variable number of hydranths and gonangia.

The preceding section has shown that the growth of these is marked by a succession of definitive structural states. And it has been shown that progression from one state to the next may be taken as expressing the dominance of a basic growth activity. Thus progression from bud to cone is expression of proliferation dominance; progression from cone to cylinder is expression of differentiation dominance; and so on similarly.

If the developmental status of each hydranth and gonangium in a collection of colonies is recorded at beginning of culture, and again after a given interval, say 24 hours, it will be found that many have changed.

Immature members will have progressed towards and to maturity; mature members may have regressed to senility and beyond; and new members will have appeared, not only *de novo* from anlagen, but also by recurrence as replacements of initially present members which have undergone catabolic dissolution.

The changes undergone by each member will have followed the sequence outlined in the preceding section. Buds will have gone on

to cones and beyond; cones will have gone on to cylinders and beyond; and so on similarly.

So the extent to which any given activity is expressed may be found as follows. Record is made of the initial number of organisms at a given structural state and of the number of these which have gone to or through the next state during the period of culture. The latter divided by the former gives the percentage of organisms which have expressed the particular growth activity subtended by the change.

An example will clarify. If a collection of colonies has 108 buds at beginning of culture, and 76 of these go on to or through cones within 24 hours; then 76/108 or 70 per cent is the extent to which proliferation was expressed by the initially present buds during the stated period. In other words 70 per cent of the potentiality for proliferation came to expression. And so on similarly for other expressions and activities.

But Obelia growth is not a series of hops from one distinctive structural state to the next. Rather is it a gradual unfolding. As a result structures intermediate between the definitive states are frequently encountered.

This being so it is obviously necessary to decide whether such intermediates belong to antecedent or succeeding states so that consistency may be had in computing the percentage progression.

Structural criteria for such decisions have therefore been established. Their description, rationale, and use in practice are the subject of this section.

Initiation

The presence of anlagen is evidence of initiation activity. But the use of anlagen to measure initiation expression is not feasible. In the first place not all of what seem to be anlagen turn out to be such. At least they produce no buds. In the second place not all anlagen are detectable without time-consuming intensive scrutiny. And finally, and most important, most anlagen which develop during the period of culture go on to form the colonial elements they subtend before the final record is made, so they are not found as anlagen but only as their products.

Now every bud is easily recognized, and buds are the product of antecedent preparatory or growth initiation processes. Since then bud

emergence is clearly evidence of initiation activity, bud emergence is taken as the mark of initiation expression.

A positive error is of course introduced when initially present anlagen are not noted, but only the buds derived therefrom during culture. This is because the initiation activity productive of such buds took place before exposure of the colony to experiment.

But a negative error is also introduced. This comes from the fact that no note is made of anlagen present as such at end of culture. For here initiation activity has been expressed under experimental conditions but not recorded.

The two errors tend to balance each other. Furthermore the residuum from this balancing is percentagely negligible in terms of total initiation activity. For it only applies to New hydranths and gonangia, and there are usually few in number in comparison with the hydranths which recur from pedicels on which other hydranths have previously functioned.

We can now consider the distinguishing marks of bud emergence.

Bud emergence from hydrocaulal anlagen is marked from the beginning by an outward bulging. No bulge: no bud (Figure 1 vs. Figure 2 of Plate 1). The bud bulge is distinguishable from the annulation bulge on several counts. In the first place it is localized on one aspect of the hydrocaulus and is not circumferential in character. In the second place it is close-packed with cells in fan-like arrangement, while the cell population of the annulation bulge is sparse, discontinuous, and interspersed with large cell-free areas (Figure 1, Plate 5). And finally, if the bud is just beginning, the underlying anlage is so characteristic nothing else can be mistaken for it. These distinctions hold not only for bud beginnings in the distal hydrocauli; but also for those in the region of pedicular jointure.

It is difficult however to distinguish between beginning hydranth and gonangial buds. Buds arising from hydrocaulal anlagen in the upper two-thirds of large colonies never turn out to be gonangia, at least in *Obelia geniculata*. But buds arising in the basal reaches may turn out to be gonangia more often than hydranths. The only way to tell is to wait and see what they grow into.

On the other hand slightly older gonangial buds can be distinguished from hydranth by the trained observer in most cases. For the outermost growing section of the gonangial bud is flatter than

that of the hydranth and is more like a tam-o-shanter than a ball. But even here sometimes what seems to be a gonangial bud may turn out to be a hydranth. This infrequent occurrence suggests the possibility that a bud might start out as a gonangium and then change into a hydranth. Dr. Theodore Hauschka is investigating the matter.

Bud emergence from ends of pedicels on which previously existing hydranths have undergone natural regression and dissolution is somewhat different. Here outward bulging from the end of the pedicel is not a decisive mark of bud beginning. For there is already present a slightly rounded eminence left by the sealing in of the pedicel after the old hydranth has been resorbed back into the colony.

What is decisive is the transition from the yellowish cast of the older terminal annulation to the paler more translucent appearance characteristic of young tissue, of the expanding roundness, and a seeming greater turgidity of fullness within the expansion.

In other words, the mark of bud emergence here is the development by the outward bulging of an appearance of newness and lability which is distinct from the set and stable appearance of the older pedicel from which it grows.

A slightly different mark is used for bud emergence from the ends of pedicels broken by the vicissitudes of living. Here healing after injury produces no outward bulge. Instead the terminal plate is either flat across the end of the distal annulation, or has the concavity of a shallow saucer.

By this concavity or this flatness is the broken pedicel recognized, and it is from this that regeneration gives rise to hydranth bud emergence.

The mark of beginning bud here is simple. No outward bulge: no bud. And like others the bulging bud is close-packed with cells in fan-like arrangement, and its pale newness is sharply distinct from the swarthy rigidity of the old annulations of the pedicel from which it grows.

The remaining site of bud emergence is from the cut end of the basal hydrocaulus. This heals over to seal in the stalk with a saucer-shallow slightly outward-curving plate stretched clear across the cut-off surface. The mark of beginning stolon bud is a rounding out of this plate as an extension of the coensarcal tube, with recognizable

beginnings of stolon-characteristic walls, free from hydrocaulal adventitia.

From the foregoing it is evident there are four distinct types of initiation activity in Obelia colonies in culture, viz.—

1. Initiation *per* hydrocaulal anlagen. This is called New Growth because it is the basis of colony population increase. It has two expressions,—hydranth production and gonangium production. Though there is a common reaction basis, for both, there are participants, determinants, and reactions in each which differ from those of the other. Otherwise the products would not differ. In other words there are reactions specific to hydranth origin from hydrocaulal anlagen, and reactions specific to gonangium origin.

2. Initiation *per* pedicular restoration. This is called Recurrent Growth because its hydranths are replacements of animals which have grown, lived, died, and naturally passed away on the same origin. It has three expressions—hydranths from initially present complete animals, hydranths from initially present senile animals, and hydranths from initially present empty hydrothecae. Since each is a sequence of the same processes, viz., hydranth regression and catabolism, and since all are hydranths, no specific distinction between expressions seems present.

3. Initiation *per* pedicular reconstitution. This is called Regeneration because it is re-production of organism after loss from injury. Since the underlying processes are in part repair processes, the type is not quite the same as recurrent growth initiation. It has but one expression.

4. Initiation *per* stolonization. This is called Vasculogenesis because it yields extension of the channel through which materials for substance increase are distributed to non-feeding members. It has but one expression.

Thus initiation has seven expressions. Individually and collectively they are of practical use in differential analysis of the part played in growth by any compound or procedure.

Proliferation

Although one mostly sees hydranth buds as in Figure 3, Plate 1, and cones as in Figure 1, Plate 2, intermediates like that of Figure 4, Plate 1, are sometimes encountered. The practical problem is to

decide whether the latter are to be put with the buds or cones in computing the percentage expression of proliferation activity.

It is our procedure to put all early hydranth states which have curved profiles into the bud group, and to reserve straightsidedness for the cones. No straight profile: no cone.

We do this despite the fact proliferation dominance is expressed in the small change from Figure 3 to Figure 4 of Plate 1 for the following reasons.

Interest is in what happens during culture. Therefore to group intermediates as buds at beginning of culture is deemed proper because such express proliferation to much greater degree during the period of culture than they have up to the time of initial record. And to group intermediates as buds at end of culture is deemed proper because such have not expressed during culture any but the beginnings of proliferation dominance.

Furthermore any negative weighting which might occur from the latter procedure tends to be balanced by positive weighting accruing from the former. Combining this with the fact the percentage of intermediates in the totals of buds and cones is small, it becomes clear that the classification entails no serious distortion of proliferation expression measure.

There are six expressions of hydranth proliferation *per* conification in Obelia colonies in culture. Cones evolve from buds derived from initially present complete and senile hydranths, empty hydrothecae, and broken pedicels. They derive from initially present buds, and from New buds originating in hydrocaulal anlagen. Since the nature of the reaction basis is presumably the same for all these expressions, there is presumably but one type of hydranth proliferation dominance.

While the gonangial situation is a little fuzzy around the edges, the core of the decisions from structural states is sound enough to be useful.

The first product of the gonangial bud, like that of the hydranth is an annulated stalk. Although proliferation is involved, this extension is not used as a mark of such activity for reasons similar to those stated in the discussion of pedicular growth.

But here the similarity ends in so far as structural changes are concerned. The distal segment of the annulated gonangium changes into a flat essentially non-productive disc: while that of the hydranth

proceeds to produce a feeding animal. The annulated gonangium becomes a chamber wherein the medusae develop. The hydranth on the other hand grows outside and on top of its pedicel. And marked changes in size and contour of the gonangium occur coterminously with medusal growth. But hydranth growth is unaccompanied by any such phenomena in the pedicel.

Despite these differences the same succession of basic growth activities is responsible for gonangial growth as for hydranth.

The practical problem here as there is to determine what structural states may reasonably be taken as marks of separation of one activity expression from another. In the hydranth this was simple because all we had to consider was the growing animal. In the gonangium it is not so simple because we have to consider gonothecal as well as medusal states. In fact we have to depend largely on gonothecal states save at the beginning. For the expression of each growth activity in the medusae so melds into its successor under gross examination that structural marks of where one may be presumed to leave dominance and another take over are not easily detectable. The gonotheca, on the other hand, do exhibit sharp and distinctive changes in structural state, and since these are presumably subtended by change in medusal growth, their use seems admissible. This does not mean that the attainment of distinctive structural states by the medusae during their growth progression is not exhibited. Far from it. What it does mean is that they pass almost insensibly from one distinctive state to the next, and so insensibly, that lines of demarkation are hard to draw on a structural basis.

With this foreword we can turn to the structural state used to mark the separation of initiation from proliferation dominance in the gonangia.

The annulated bud is a tight-knit slightly conical structure, surmounted by a flattish outward-curving cap, and within which no medusae are detectable. Shortly an over-all lengthening combined with a progressive distal broadening brings the gonangium to the form of a wide squat cone. The terminal segment subsides into a broad disc which seals the contents from the outside. The medusal mass emerges into recognizability, and clearing spaces appear between this and the flattening-out annulations.

Since no medusae are detectable before these changes occur; and

since the primary function of these changes is aimed at medusal production; and since the appearance of medusal growth is presumably dominated by cell increase in number; all early structures which show no medusae are put in the bud group, and those which show the first faint shadows of medusae are put with the cones. No medusae—no cone: medusae no bud. This then is the structural difference used to mark the separation of initiation from proliferation dominance in the gonangia.

Two expressions are given, viz., cones from initially present buds, and cones from New buds originating from hydrocaulal anlagen during the period of culture. Since the reaction basis is presumably the same for each, there is presumably but one type of gonangial proliferation dominance.

But the gonangial reaction basis is clearly different from the hydranth since the product thereof is different. Therefore Obelia colonies in culture may be deemed to express two types and eight expressions of proliferation activity dominance. Individually and collectively these are of practical use in differential analysis of the part played in growth by any compound or procedure.

If the reader is not comatosed by now, he will ask: Why is not the proliferation activity of the stolon included in this array of expressions?

The formation of a hydranth bud on a stolon tip is not a change from no proliferation to proliferation; but a change from stolon growth to hydranth growth which is not proliferation but a type of cell specialization or differentiation.

And stolon growth in length is not used any more than pedicular growth in length, or hydranth cone expansion; first because of the unmeasurable contribution of hydroplasmic pressure; second because size increase here as elsewhere is largely an index of constructive substance increase; third because it is not convenient; fourth because it is not necessary; and fifth because once a stolon is started it grows in length under test as under control conditions. Hence proliferation dominance is expressed in both and no opportunity for distinction is had. This last point is also a reason why pedicular growth in length is not included in the constellation of proliferation expressions.

Differentiation

The first structural indication that cell specialization is about to take over from cell increase in number is the disassociation of the hydranth cone from the hydrotheca in its basal third. This postulate is confirmed by physiological evidence. For Hauschka is reporting that disassociation is concomitant with the coming to expression of an *ipse facto* gastric tissue motility which is distinct from that imposed by surge and recession of hydroplasmic pressure. Hardly anyone would claim this assumption of a hitherto unexpressed and independent property is not evidence of cell specialization or differentiation, and *pari passu* that the accompanying structural change is not similarly indicative.

Despite this these structures are put with the cones (Figure 3, Plate 2) and not with the cylinders (Figure 3, Plate 1) in computing the extent to which differentiation is expressed by the method of percentage progression.

This is because the first disassociations are not visibly maintained. It is frequently found that a separation seen at one time is not seen half a minute later. Instead the hydranth is found replastered against the hydrothecal wall. This flapping back and forth of the basal tissue derives of course from shifts in hydroplasmic pressure and the natural rhythmicity of the developing hydranth. Such a situation makes for uncertainty. However, the errors introduced by putting these intermediates with the cones balance out here just as they do in the case of the bud intermediates. And for the same reasons.

So a slightly more advanced state is used as the structural mark of separation between proliferation and differentiation dominance. As differentiation advances a bit, a ring of basal hydranth tissue remains fixed to the hydrotheca just above the pedicel. Constrictions below and above this circle of attachment help to make of the structure a little shelf. This is shown in Figure 4, Plate 2, and succeeding photographs. This is permanent during the life of the animal. So shelf appearance is taken as the mark of separation between cone and cylinder. No shelf—no cylinders: shelf—no cone.

On this basis there are seven expressions of hydranth differentiation exhibited by Obelia colonies in culture. There are cones derived from initially present buds, complete hydranths, senile hydranths, empty hydrothecae, and broken pedicels which go on to

cylinders. There are initially present cones which do likewise, and so do cones produced in the course of New hydranth growth from hydrocaulal anlagen. Since all these presumably have one kind of reaction basis they may be taken to represent a single type of cell specialization.

Another type is that exhibited in stolon metaplasia. Here the stolon, instead of continuing its growth as such, produces a hydranth from its growing tip. The first mark of separation is either a beginning annulation, or a beginning hydranth-characteristic bud. No annulation or no bud: no metaplasia. But one expression of this type of cell specialization is customarily found. When such metaplasic hydranths progress from cone to cylinder they express, of course, the same type of differentiation dominance as all hydranths and may be put in the group described above.

As far as the gonangium is concerned, dependence is almost exclusively upon gonothecal structure. Here differentiation is taken to be entering on dominance when the distal sealing-in disc, instead of being the base of an inverted cone, becomes the shrunken stopper of the constricted mouth of an outwardly tapering sac. So the structural line of demarkation between proliferation and differentiation dominance for purposes of computation is taken to rest on decision as to whether the distal seal is the base of a cone or the plug of a bottle.

This is not very satisfactory, but it is the best that can be done under gross examination. Fortunately intermediates are rarely encountered. Mostly all that is seen is either a well-defined cone, or an elongated bulging sac, the narrowed distal opening of which is closed by a short cylindrical plug, and within which the medusae are distributed along the stretched-out blastostyle like translucent beads on a chain instead of being clumped together like a bunch of grapes.

Three expressions of this dominance may be given by gonangia during a 24-hour period of culture. Sacs may develop from cones derived from initially present buds, and from cones produced in the course of New Gonangium growth from hydrocaulal anlagen. And such may develop from initially present cones.

The same kind of reaction basis may be deemed to serve all three expressions. But the type exhibited here obviously differs from that

concerned in stolon metaplasia and hydranth growth since the products differ.

So all in all there are 11 measurable potentialities of differentiation expression in Obelia colonies in culture, and three types of measurable activity. Individually and collectively these may be put to practical use in evaluation of the part any compound or procedure may play in growth.

Organization

The hydranth change in contour from Figure 1 to Figure 2 of Plate 3 is not expression of Organization dominance. It is simply reflection of the independent contractility of the presumptive gastric tissue, aided and abetted by hydroplasmic pressure change.

In Figure 3, however, things are different. Here the stomach is shaping up; the distal cell mass is a thinner section of a cylinder; and hint of separation of the crown,—which is to form the mouth,—from the rim, from which the tentacles are to come,—is evident. Further there is seeming separation from the hydrotheca at one edge.

The combination suggests that segregation of the cells (or organization) into the definitive structures characteristic of the completed animal has begun.

Despite this, such structures are put with the cylinder group for computation. The reason is practical. Namely, that changes in internal pressure tend to erase now one and now another of these criteria, and thus make decision difficult on gross examination. The errors introduced by this procedure tend to balance out as they do in the case of bud and cone intermediates, and for the same reasons.

In practice, then, we do not use this as the mark of separation of completes from cylinders, but the first visible separation of cells into the beginnings of recognizable tentacles. No tentacles—no complete: tentacles—no cylinder. Sometimes the first sign of tentacles is a shadowy vertical parallelization within the distal cell mass; and sometimes it is a knobbing out of one or more small ridged transparencies from its upper surface. Figure 4 of Plate 3 is a poor showing of the distinctions.

Nine opportunities for expression of organization dominance in hydranths are presented by Obelia colonies in culture. Complete animals evolve from initially present cylinders, and from cylinders de-

rived from initially present buds, cones, senile hydranths, empty hydrotheca, and broken pedicels. They also evolve from cylinders produced during the course of New Hydranth growth from hydrocaulal anlagen. Given time such can also be had from initially present complete hydranths, and hydranths developing at the end of stolons by metaplasia. Since the reaction basis is presumably the same for all expressions of hydranth organization into the completed animal, these represent but one type of organization activity.

As far as the gonangium is concerned the structural mark of distinguishment is the opening of the distal end of the gonotheca. Sometimes the cylindrical plug is absent. Sometimes it remains attached though pushed aside. Sometimes few medusae have swum out of the sac, sometimes many, or all. Never have we happened to find fully developed medusae swimming within an unopen sac. This does not mean that such may not be found by others. If so, the natural procedure is to consider that organization has been expressed, and class the structure accordingly. But in our experience the practical mark of distinction between differentiation and organization dominance is the open gonotheca. For, by and large, this means the medusae have completed their developmental growth and are ready for egress.

Four opportunities for such expression may be found in Obelia colonies in culture. Open gonotheca may evolve from initially present closed sacs; from closed sacs derived from initially present buds and cones; and from closed sacs produced during the course of gonangial growth from hydrocaulal anlagen. Since the reaction basis for change from closed to open gonotheca is presumably the same wherever it is expressed, the four expressions here noted may be deemed to represent but one type of organization activity. This type, however, is deemed different from that concerned in hydranth organization, since the products are different.

Thus Obelia colonies in culture may present 13 expressions and two types of organization dominance which may be put to practical use in evaluation of the part played in growth by any compound or procedure.

Constructive Substance Increase

Size changes are not a very good index of mass growth in Obelia for three reasons. First—dimension is factored by change in hydro-

plasmic pressure; second—all volume change is not substance increase; third—all volume is not Obelia substance; and fourth—hundreds of measurements would have to be made in each colony, and when thirty colonies are used in a single experiment, as is our custom, the time required would militate against reliability, since growth in these colonies is continuous.

This does not mean that dimension measurements have no proper place in studies of this material. They do, of course. And there is no need to recite here where they can and should be used. But for our purpose they are as unnecessary as they could be misleading.

What we want to know is whether or not a given compound acts specifically on constructive substance increase as distinct from any of the other basic growth activities. And a fairly reliable index of this can be obtained by determining whether or not colonial growth as a whole exceeds, falls below, or is the same in test cultures as in control.

In practice, then, the mark of constructive substance increase is the sum of the growth progressions from one structural state to the next.

It need hardly be stated that the rationale of this procedure stems from the fact that this growth activity is a usual accompaniment of the others.

Decision, however, is subject to checks and balances emanating from test differences from control in expression of the correlative activities of Maintenance and/or Regression and Catabolism. Examples may be found in preceding reports (196:197). Exposition in greater detail will be given in Part II of this Monograph.

Maintenance: Regression

The dominant activity expressed by the complete hydranth is Maintenance. This eventually dies away and the hydranth undergoes a structural regression which merges into the senile state. There is no well-defined structural state characteristic of a hydranth in a state of regression. But there are structural criteria distinguishing senile hydranths from completes. So the measure of regression is the percentage of initially present completes which go on to the senile state during a period of culture. And the measure of maintenance is the percentage of initially present completes which stay as such during the same period.

While the first sign of regression is withdrawal of the hydranth within its hydrotheca, withdrawal as such is not necessarily a sign the animal is permanently losing its maintenance function.

Retraction occurs in response to various stimuli; occurs when the animal is not feeding; may be correlated with tidal rhythm; and may be simply expression of resting. From all such there may be re-emergence, and resumption of function.

Because of all this, beginning senility is of necessity taken as the mark of regression from maintenance.

The structural criteria for separation of beginning seniles from completes then, is not shrinkage within the hydrotheca as such (Figure 2, Plate 4); but this plus evidence of loss of structural integrity in the contracted hydranth.

In practice the separation is based on the inability to detect any tentacular structures, save possibly a tag end or two sticking shortly out at the top; or an appearance of einschmelzung in the hydranth mass; or both. A fairly advanced state is shown in Figure 3 of Plate 4. Thus: no tentacles—no senile. No senile—no regression; but maintenance.

While this procedure tends to weigh the values towards maintenance persistence, the error is slight because the number of seniles in the beginning state is usually small. Furthermore, when progression in one group (test) is being compared with that in another (control), this or any other weighting is of little moment, since the same error is introduced in both groups. And interest is primarily in the direction and not the amount of deviation of one from the other.

Obelia colonies in culture exhibit but one type and one expression of hydranth maintenance and/or regression dominance.

Catabolism

Since a senile hydranth is evidence of catabolic activity, a good measure thereof would be the percentage of hydranths which go on to seniles from the immediately antecedent state. But this is the state of regression. But there is no well-defined structure by which the hydranth can be distinguished from the completes on the one hand, and the seniles on the other. So such procedure cannot be used. Nor can the progression from complete to senile hydranths. For this is pre-empted by Regression. But it is desirable to separate

this activity from that of catabolism, since each is determined by a different set of chemical reactions. So the percentage progression from senile hydranths to empty hydrothecae is used in practice as the measure of catabolic activity. Such procedure gives the extent to which catabolism is completed during a given culture period. And the result is an adequate index for determination of whether or not catabolism in test cultures differs from that in control. Which is what we want to know.

Now an empty hydrotheca is indication that catabolic dissolution of the previously existing hydranth has been accomplished. Most empty hydrothecae are truly empty. But some contain hydranth residues. Such are not taken to be empty unless nothing is left of the hydranth save a small ball on a thin stalk through which there is no longer communication with the pedicular hydroplasm. Such is pictured in Figure 4 of Plate 4.

All structures which present hydranth tissue still in the process of dissolution, and with a patent stalk, are put with the seniles. Those which have none such are put with the empties. There is little chance for error if the observer asks himself: is hydranth catabolism completed?

There are two expressions of this single type of catabolism dominance in Obelia colonies during a 24-hour culture period. Empty hydrothecae come from initially present senile hydranths, and from seniles derived from initially present complete hydranths.

Thus ends exposition of the practical bases for allocation of intermediate structural states to definitive stages in the life-cycle of Obelia hydranths.

Succinctly, the procedure is based on thinking of each structural state, not as fixed and static, but as the product of antecedent definitive processes; as representing at the moment of observation, either the end-result of the immediately antecedent dominant activity, or the coming to dominance of the succeeding activity; and as going on from the moment of observation to express by other distinctive structures the successive dominance of other definitive processes.

In fact the development of this way of thinking about growth, not only in Obelia, but also in all other growing things, could do much to bring about that integration of its interdependencies, which is

essential to understanding of the phenomenon as a fundamental property of all nature.

I have now done what I set out to do; namely to demonstrate that the colonial-living form of the marine hydroid Obelia is potentially suitable test material for determining whether or not any given naturally occurring amino acid and nucleic acid component of general distribution is specifically important to any given basic growth activity.

For I have shown that the Obelia hydranth meets all the tests for suitability, in that it exhibits the basic growth activities in such fashion the dominant expression of each is distinguishably separable from that of the others in such manner the extent thereof may be measured with reasonable accuracy. And in that the animal submits gracefully to laboratory culture; derives is nitrogenous subsistence from pre-formed amino-acids; and is sufficiently abundant for extensive experimentation.

In conclusion let me warn the uninitiated not to take from what is here set forth the idea that all these structural distinctions, separations, and allocations are claimed to mean an equivalent singleness in the activity of which they are the product. They are simply to be taken as markers either of dominance of a given activity, or of transition from one dominance to another. Only that and nothing more. As such they serve as practical indicators for measurement of the extent to which progression from one dominance to another is accomplished.

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RELATIVE GROWTH OF THE EUROPEAN EDIBLE CRAB, CANCER PAGURUS: III. GROWTH OF THE STERNUM AND APPENDAGES

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A. INTRODUCTION

In this third paper on the relative growth of the European Edible Crab, *Cancer pagurus*, the growth of the sternum and appendages is considered in some detail. The first paper in this series (MacKay, 1942) dealt with the growth of the carapace and the second (MacKay, 1943a) with the growth of the abdomen. All three papers are the outcome of an investigation of the relative growth of *Cancer pagurus* carried out by the writer in the United Kingdom during the year 1934-35. The study was conducted at the Laboratory of the Marine Biological Association of the United Kingdom and was made possible by the granting of a Royal Society of Canada Fellowship in Zoölogy for that year. Since the methods of collecting, measuring, and analyzing the data have been reported already in the first paper of the series this information will not be repeated here.

B. THE MEASUREMENTS STUDIED

All measurements used in this study were made by the writer with a Glogau sliding jaw caliper calibrated in centimeters and all crabs were caught in the vicinity of Plymouth, Devonshire, England. All deformed, regenerating, or soft appendages were rejected. As in the earlier papers the parts studied are designated by letters. The carapace widths were represented by the letters A to H inclusive, the abdomen widths by the letters I to O, and here the sternum widths are represented by the letters P to S and the appendages by the letters T to Z.

The following are the dimensions of the sternum and appendages to be considered in this paper (see also Figure 1):

1. Sternum width P (see Figure 1 for location).
2. " " Q (" " " " ").
3. " " R (" " " " ").
4. " " S (" " " " ").
5. Chela length T (maximum length of the manus or hand of right chela).
6. Chela width U (maximum width of the manus or hand of right chela).
7. Periopod length W (maximum length of 1st peripod on right side).
8. " " X " " " 2nd " " " ").
9. " " Y " " " 3rd " " " ").
10. " " Z " " " 4th " " " ").

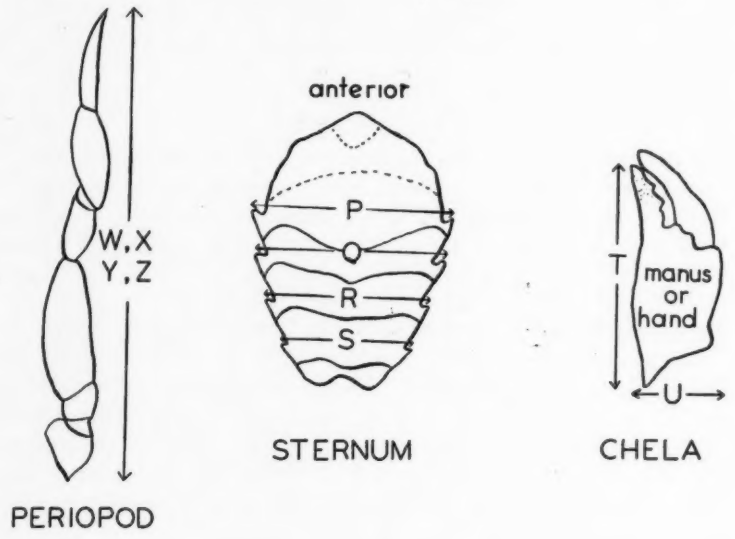

FIGURE 1
DIAGRAMS OF THE STERNUM AND APPENDAGES SHOWING THE DIMENSIONS STUDIED

The present portion of the study is based on the analysis of 6,856 measurements, 3,473 for males and 3,383 for females. With the data herein presented 26 body measurements will have been studied in relation to carapace length by Huxley's well known method (Huxley, 1932). For this study to date 16,745 measurements have been analyzed, 8,446 for males and 8,299 for females.

C. RELATIVE GROWTH OF THE STERNUM

The results of plotting the various sternum dimensions are sum-

marized in Table 2 and are graphically represented in Figure 3. It will be noted that with the exception of the most posterior dimension (S) no significant relative growth differences based on sex have been found for the sternum of immature individuals. In the antero-posterior axis there is indication of a small increase toward the posterior in the relative growth constant.

In mature crabs the males closely resemble the immature condition in so far as the constant k is concerned. Males and females differ markedly only for dimension S, the most posterior of the sternum widths, the females having a higher growth ratio. With increasing body size the higher differential growth ratio for this dimension would lead to the production of a sternum considerably wider toward the posterior margin in the female than in the male. This may well be related to the fact that the female abdomen which adjoins it is wider than that of the male.

D. Relative Growth of the Chelae

A double logarithmic plot of the right chela length (dimension T) against carapace length gives interesting results (see Figure 2 and Table 1). For males the highest value for the constant k revealed by this study was obtained for this dimension ($k = 1.48$). Examination of Figure 2 shows that adult males have a higher relative growth constant than adult females, that for immature individuals there is little difference between the sexes in this respect, and that it is the males that change. This change in the relative growth ratio is quite marked and occurs at a carapace length of between 5.9 and 7.4 cm. It has been pointed out previously (MacKay, 1943a) that the carapace width at sexual maturity determined by Orton (1936) would correspond to a carapace length of 6.7 to 8.3 cm., a size that is probably reached in the third year. The increase in the differential growth ratio in the male chela coming as it does at the size at which sexual maturity is attained is almost certainly related thereto, and the large claw of the adult male may be considered to be a secondary sex character.

While Table 1 and Figure 2 are based on the right chela many measurements were also obtained for the left chela and the two were compared. There is apparently no significant difference in size or in growth between the right and left chelae of *Cancer pagurus*. Lob-

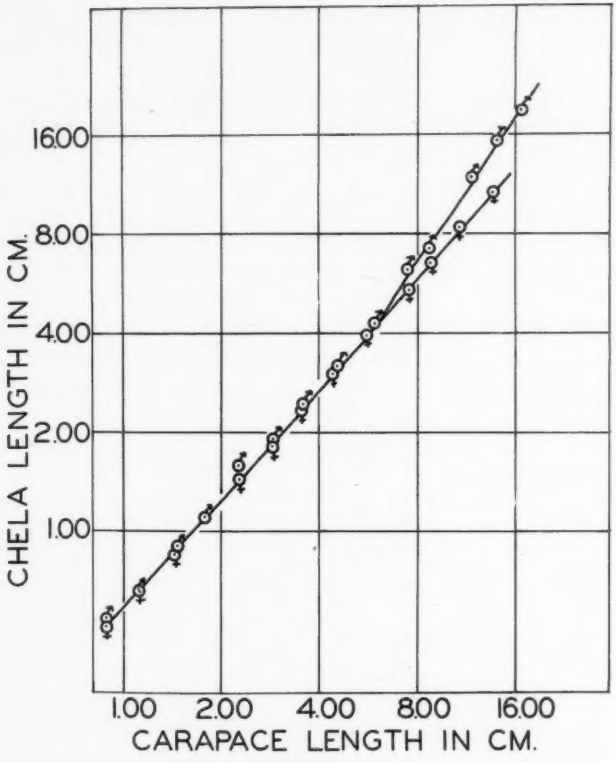

FIGURE 2
GROWTH OF THE CHELA OF *Cancer pagurus*

sters, shrimps, and many other crabs do display form or size differences in the two chelae, however.

For dimension U, the chela width, the differential growth constant for immature males and females is 1.10. At a carapace length of about 5.8-7.2 cm. both males and females show a change, the males increasing to 1.40 and the females decreasing to 1.04.

E. RELATIVE GROWTH OF THE PERIOPODS

Crabs belong to the Order *Decapoda* which derives its name from

TABLE 1

MEANS OF CHELA DIMENSIONS T AND U TOGETHER WITH LENGTHS OF CARAPACE OF MALE AND FEMALE CRABS

Lower limits of size classes	Males No.	Males Mean carapace length	Males Mean length dimension T	Females No.	Females Mean carapace length	Females Mean length dimension T
0.00	6	0.87	0.51	8	0.86	0.49
1.00	11	1.08	0.64	9	1.10	0.64
1.26	19	1.44	0.83	21	1.45	0.89
1.58	32	1.77	1.09	35	1.81	1.13
2.00	34	2.25	1.57	40	2.25	1.42
2.51	32	2.85	1.88	46	2.85	1.84
3.16	35	3.52	2.40	57	3.51	2.33
3.98	13	4.43	3.13	22	4.38	3.00
5.01	11	5.79	4.20	12	5.46	3.83
6.31	61	7.38	6.10	47	7.46	5.42
7.94	60	8.72	7.08	71	8.74	6.37
10.00	37	11.49	11.75	15	10.81	8.12
12.60	48	13.93	15.05	11	13.46	10.45
15.80	4	16.47	18.61			
Total	403			394		

Lower limits of size classes	Males No.	Males Mean carapace length	Males Mean width dimension U	Females No.	Females Mean carapace length	Females Mean width dimension U
0.00	6	0.87	0.24	8	0.84	0.23
1.00	11	1.08	0.31	9	0.97	0.32
1.26	19	1.44	0.47	21	1.45	0.43
1.58	32	1.76	0.54	35	1.81	0.55
2.00	34	2.25	0.70	40	2.26	0.70
2.51	32	2.85	0.91	46	2.85	0.89
3.16	34	3.52	1.15	56	3.51	1.14
3.98	13	4.43	1.49	22	4.38	1.43
5.01	11	5.79	1.99	10	5.52	1.87
6.31	61	7.38	2.94	47	7.46	2.57
7.94	60	8.72	3.82	71	8.72	3.02
10.00	37	11.49	5.73	15	10.81	3.82
12.60	48	13.93	7.33	11	13.46	4.92
15.80	4	16.47	9.01			
Total	402			391		

the fact that its members possess ten legs arranged in five pairs. The first or most anterior pair consists of the chelipeds or large claw-appendages. The chela or claw portion of the cheliped has been considered in the preceding section. The remaining four pairs of legs are used for walking and are essentially similar in appearance.

An analysis of the relative growth of these four pairs of appendages

TABLE 2

SUMMARY OF THE RESULTS OF PLOTTING THE VARIOUS STERNUM AND APPENDAGE MEASUREMENTS

Part	Dimension	Remarks
Sternum	P	For immature crabs k has a value of 1.05 for males and of 1.06 for females. k is 1.06 for mature males and 1.03 for mature females. The change occurs in males at a carapace length between 5.8 and 7.3 cm. and in females between 5.4 and 7.4 cm.
"	Q	k is 1.06 for immature and mature males and 1.05 for immature females. For both sexes there is indication of an earlier change in this constant, crabs below about 1.5 cm. having considerably higher differential growth ratios. k for mature females is 1.03, the change from 1.06 occurring at a carapace length of between 5.5 and 7.5 cm. There is a change in the constant b for males between a carapace length of 4.5 and 5.8 cm.
"	R	For males of all sizes above 2 cm. k is 1.09; for females above the same size it is 1.10. Crabs of both sexes below about 2 cm. appear to have somewhat higher growth ratios.
"	S	For immature crabs k is 1.08 for males and 1.14 for females. For mature males $k = 1.06$ and for mature females $k = 1.17$. The change occurs in males at a carapace length of between 7.3 and 8.8 cm. and in females between 5.4 and 7.4 cm.
Chela	T	The k for males and females is 1.12 until a carapace length of 5.9-7.4 cm. is reached. Above this size the females show no change but the males change from 1.12 to 1.48 the latter value being the highest found for any dimension of the males of *Cancer pagurus*. See Table 1 and Figure 2.
"	U	k has a value of 1.10 for both sexes up to a carapace length of 5.8-7.2 cm. at which size it decreases to 1.04 in females and increases to 1.40 in males. See Table 1.
Periopod	W	For the first walking leg the young of both sexes have a k of 1.05. At a carapace length of 4.4-7.4 cm. k changes to 0.90 in females and at a carapace length of 4.5-7.1 cm. it changes to 1.03 in males.
"	X	k is 1.07 for the young of both sexes and for all males. At a carapace length of 5.5-7.4 cm. k changes to 0.91 in females.
"	Y	k has a value of 1.08 for the young of both sexes and for all males. k changes to 0.92 in females at a carapace length of 5.5-7.4 cm.
"	Z	k has a value of 1.08 for the young of both sexes. k changes to 0.96 in females at a carapace length of 4.4-5.5 cm. and to 1.0 in males at a carapace length of between 4.4 and 7.4 cm.

reveals certain facts of interest. It will be recalled that the chela had a very high relative growth ratio (this does not necessarily mean that the whole cheliped has a high k, however). The remaining four pairs of legs have a much lower differential growth ratio and it is lower for adult females than it is for adult males (see Table 2); for immature individuals no sex difference could be observed. The difference in relative growth ratio between adult males and females results mainly from a *decrease* in the ratio for adult females whereas the difference in the chela length was found to result from an *increase* in the adult males. Large adult female crabs come to have considerably shorter legs as well as smaller chelae than adult males.

F. Relative Growth and Sexual Maturity

Huxley's observation that changes in the relative growth of animals are frequently correlated with the onset of sexual maturity is substantiated by the findings of many workers. A number of changes in the relative growth of *Cancer pagurus* have been found to occur at or near the size (6.7-8.3 cm. carapace length) at which Orton (1936) finds that the species probably becomes mature (see also MacKay, 1943a). In addition to the changes previously reported by this writer certain additional ones are revealed by the present portion of the study. These are as follows:

(1) The k for sternum width P changes slightly in males between 5.8 and 7.3 cm. carapace length and in females between 5.4 and 7.4 cm.

(2) For dimension Q k changes slightly in females between 5.5 and 7.5 cm. carapace length; in males there appears to be a small change in the constant b between 4.5 and 5.8 cm. carapace length.

(3) Females show a change in k for dimension S at between 5.4 and 7.4 cm. carapace length; males show no corresponding change.

(4) For dimension T (chela length) there is a marked change in k at a carapace length between 5.9 and 7.4 cm.; females display no corresponding change.

(5) For dimension U (chela width) there is a large increase in the value of the constant k for males and a small decrease for females at a carapace length of 5.8-7.2 cm.

(6) The k for periopod dimension W shows a change in males at between 4.5 and 7.1 cm. and in females at between 4.4 and 7.4 cm. carapace length.

(7) The k for periopod dimension X changes in females at between 5.5 and 7.4 cm. carapace length.

(8) The k for periopod dimension Y shows a change in females at a carapace length between 5.5 and 7.4 cm.

(9) The k for periopod dimension Z shows a change in males at a carapace length between 4.4 and 7.4 cm. and in females between 4.4 and 5.5 cm.

Therefore, the total number of changes in the differential growth constant found to occur at the size (and consequently at the time) of sexual maturity in the various carapace, abdomen, sternum, and appendage dimensions of *Cancer pagurus* is considerable.

G. Gradients of the Sternum and Appendages

The growth coefficients of the sternum listed in Table 2 are shown graphically in Figure 3. Examination of this figure shows that with the exception of the most posterior sternum width (dimension S) there is little difference between the sexes and that there is some evidence, especially in females, of an antero-posterior gradient that is low at the anterior and highest for the most posterior width studied. This latter is in agreement with the carapace gradients of the dorsal surface in which a gradient highest toward the posterior has been demonstrated (MacKay, 1942 and 1943).

Of all parts and dimensions studied the length and width of the chela (dimensions T and U) have the highest growth coefficients. Unfortunately no data are available for the cheliped as a whole but there is reason to think that the k for the length of the whole appendage would be smaller than that for the chela alone.

From Table 2 and Figure 3 it will be noted that the differential growth ratios for all four walking legs (dimensions W, X, Y, and Z) are almost identical for immature crabs; however, a slight gradient increasing toward the posterior can be observed. For mature crabs there is a considerable and consistent difference in the differential growth ratios of males and females, the males having the higher ratios. For females this is in the form of a gradient which increases toward the posterior. For males the third periopod is highest and the fourth is lowest. The differential growth ratios for all four periopods are much lower than that for the chela length (dimension T).

H. Sexual Dimorphism

A marked difference in some of the growth coefficients of the two

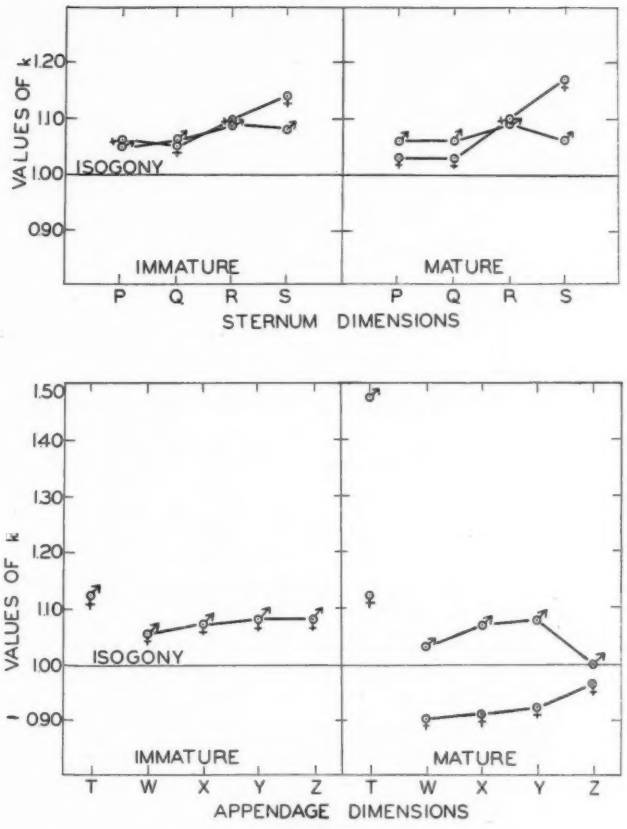

FIGURE 3

GROWTH GRADIENTS OF THE STERNUM (ABOVE) AND APPENDAGES (BELOW) OF *Cancer pagurus*

The differential growth ratios of nine dimensions are represented graphically.

sexes has been pointed out previously in this series; these differences lead inevitably to differences in the form of the abdomens of males and females. The present findings furnish additional evidence of sexual dimorphism in this species. The fact that the growth coefficient for sternum width S is considerably higher for females than it is for

males leads to a greater widening of the posterior portion of the sternum in females. This is perhaps not surprising in view of the fact that mature females display much higher relative growth than do the males for the adjoining abdominal segments.

The present study further shows that while no difference in the size of the chelae and periopods occurs before maturity, considerable differences are noted in the size of these structures for the larger individuals, the mature males having longer and wider chelae and longer periopods.

I. Heterogony and Maximum Size

The work of Weymouth and MacKay (1936) indicates that another member of this genus, *Cancer magister,* is conservative in its form changes. In other words the differential growth ratios are nowhere extremely high or extremely low. To reach this conclusion eight important body dimensions were studied. The differential growth ratios were found to range between 0.93 and 1.61 using carapace width rather than length as the standard of comparison.

The range of k for *Cancer pagurus* revealed by the present series of studies is 0.70 to 1.50. Both investigations are in agreement in indicating the small degree of heterogony in *Cancer*. Values as low as 0.32 and as high as 3.05 have been pointed out by Huxley (1932) and Shaw (1928) for other species. The effects of heterogony are cumulative and continued high heterogony will inevitably produce great changes in form such as large appendages which may hinder further growth in body size and prove a hindrance to survival (Huxley, 1932 and Weymouth and MacKay, 1936). Animals like *Cancer pagurus* or *Cancer magister* in which the growth ratios are not extreme can grow to a considerable size without great changes in form. The former species sometimes attains a weight of 10-12 pounds.

Most species of crabs are edible but few are so styled. Three factors probably make certain species important for food: (1) large size, (2) abundance, (3) nearness to markets. The genus *Cancer* is world-wide in distribution (MacKay, 1943b) and most members of the genus are fairly large. It is not surprising, therefore, that many of them are important food animals. Doubtless the large size is made possible by the low heterogony of the various parts and dimensions.

J. Summary and Conclusions

This paper is the third in a series dealing with form changes in the European Edible Crab, *Cancer pagurus*. Following the analysis of the growth of the carapace and abdomen in earlier papers the growth of the sternum and appendages are analyzed by the same methods. The data presented herein bring the total number of body measurements studied in connection with form changes in this species to 16,745 (8,446 for males and 8,299 for females).

Males and females show little difference in the growth of the sternum with the exception of the most posterior width studied; for this dimension a considerably higher differential growth ratio has been found for females. There is some indication of a gradient in the antero-posterior axis, especially in females, such that increases in width are greatest toward the posterior near the junction of the sternum and abdomen.

In both immature crabs and mature males the appendages studied display positive heterogony; in females the chela is positively heterogonic, and all four periopods negatively heterogonic. For the chela the growth coefficient is higher for mature males than it is for immature ones ($k = 1.48$ instead of 1.12). As a result of these form changes mature male crabs will come to have larger chelae (both in length and in width) and longer legs than females of corresponding body length.

For male crabs the highest growth coefficients found for the entire study (26 body measurements) have been found for the chela length ($k = 1.48$) and width ($k = 1.40$) of mature individuals. For females, on the other hand, the highest growth coefficient has been reported previously (MacKay, 1943a) for the fourth abdominal segment of the adult ($k = 1.50$). *Cancer pagurus*, like *Cancer magister*, displays a low degree of heterogony and consequently is able to grow to a considerable size without marked change in body form.

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THE EFFECT OF X-RAYS ON THE DEVELOPMENT OF INSECTS: I. IRRADIATION IN LARVAL STAGE

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The experiments reported below describe the effect of X-rays on insect larvae at various stages of their development. Insect eggs have been extensively used in radiobiological investigations. Existing data concerning the reaction of insect larvae and pupae to X-rays are, however, very meager. The earliest investigation on this subject is due to Mavor (6) who worked with Drosophila melanogaster. He was followed by Hufnagel and Jolly (4, 5) with experiments on Calliphora erythrocephala and Hyponomeuta padella, and more recently by Henshaw and Golomb (3) with an investigation on Drosophila melanogaster.

BIOLOGICAL MATERIAL AND TECHNIQUE

Experiments were carried out both on fly larvae (Calliphora erythrocephala) and mosquito larvae (Culex pipiens).

Larvae of Calliphora erythrocephala are acephalous maggots: they bear no appendages and show no differentiation into head, thorax and abdomen. Once a certain size is attained, the larval stage is at an end. The maggot then ceases to feed and becomes shorter. The alimentary tract, at first clearly visible because of its black contents, is no longer distinguishable. The maggot becomes less motile and strives to transform into a pupa. The actual process of pupa formation cannot be directly observed.

Previous to pupation the larval skin detaches itself from the body, but is not sloughed off as in ecdysis. The skin layer gradually hardens and encloses the pupa within a protective cylindrical cask, the puparium. It gradually changes in color from brownish-yellow or orange to dark brown, or almost black. When the resting pupa within the puparium has transformed into a mature imago, the pupal skin is sloughed off, and the puparium is forced open by the pressure of the protruding ptilinium. The fly is then set free.

Calliphora flies were maintained on sugar solution. After addition of a little moist red meat or liver in the diet for a number of days egg deposition occurs. After a day or two the larva hatches and burrows into the meat piece. Sometimes the meat supply for the fast growing larvae was renewed. The bottles for breeding were set up with a ground layer of sand or paper tissue into which the larvae could burrow for pupa formation. A detailed account of the rearing of Calliphora has been given by Minnich (7).

Irradiation of the larvae was carried out in glass cups (diameter 18 mm., height 10 mm.), within which 10 small-sized larvae or 5 to 6 large-sized larvae were arranged in a single layer. The cup was closed by a cellophane sheet. Test and control animals were maintained under the same conditions on a meat diet in beakers covered with gauze. Record was kept of the larval mortality and of the course of pupa and imago formation. Pupae which failed to produce flies were opened and examined.

Females of Culex pipiens lay their eggs in a batch on the surface of water. After a few days, the larvae hatch. They are about 1-2 mm. long, appendage-free, and show clear differentiation into head, thorax and abdomen. Four larval stages, clearly marked off by successive moults, are distinguished. The last—fourth—moult is attended by pupa formation. The resting larva hangs by its respiratory tube from the water surface and paddles food particles into its mouth with the aid of a ring of oral cilia. The pupa is freely motile, and unlike fly pupae, possesses no protective envelope. Like the larva, the pupa hangs at rest from the water surface by a pair of thoracic breathing tubes. After a few days of rest, the pupa skin splits dorsally at the thorax and the mosquito works its way into the air.

The experiments on mosquito larvae were carried out with Culex pipiens L. In distinction to Culex pipiens pipiens and most other Culicidae this race is able to form and lay a first batch of eggs without prior feeding on blood, if only they are provided with an abundant ration. Culex pipiens shows a marked predilection for impure nutrient-rich water (dilute hay infusion, dilute liver extract, etc.), containing an abundance of bacteria and other organisms. The animals were reared in vessels of 20 by 30 cm. sizes.

For irradiation, 10 to 15 small larvae or 6 to 8 large larvae were transferred by means of a pipette to a hollowed slide which had been

filled with water nearly to the edge, so that in different experiments roughly the same volume of water was present. After irradiation, test and control animals were rinsed into small beakers filled to about 2/3 capacity with frequently renewed nutrient solution (see above). At appearance of the pupae, the beakers were covered with mosquito nets. Larva mortality as well as pupa and imago formation were noted.

X-Ray Apparatus and Physical Measurements

A demountable X-ray tube with a copper anticathode continuously evacuated by an air pump was used. The ray emergence opening was closed with aluminum foil of 30μ thickness. The focus—object distance was 40 mm. at high doses and 50 mm. at low doses. The tube was operated at a tension of approximately 35 KV. The radiation intensity at the distance of the objects was about 2200 r/sec. at a tube current of 30 Milliampères for the high doses and for the lower doses approximately 120 and 220 r/sec. at 5 and 10 Milliampères respectively. The beam, as absorption analysis showed, consisted largely of K radiation from the anticathode (copper). The half layer value was 0.053 mm. aluminum.

Absorption measurements of pressed skin envelopes from Calliphora larvae indicated an average absorption rate of approximately 30 per cent. The proportion of radiation penetrating to the larval body was therefore 85 per cent of the incident intensity because of the absorption in the skin (approximately 15 per cent). The total absorption in large active Calliphora larvae is about 80 per cent of the incident radiation. Allowing for absorption by the skin layer a rate of about 30 per cent it follows that nearly one half the incident radiation is absorbed in the larval interior.

The skin of Culex larvae is much thinner than that of Calliphora larvae and absorption by skin may be neglected. In the experiments with Culex, allowance must be made, however, for absorption by the water in the hollowed slide. The depth of the water layer was about 1.5 mm. The larvae were generally very near the surface from which they hang by means of a breathing tube. It may be assumed that on an average the irradiated larvae are in the middle of the drop in which they are suspended, i.e., they are covered by 0.75 mm. of water. A layer of this thickness absorbs 30 per cent of the incident

radiation. The amount of radiation actually reaching the interior of Culex larvae was therefore—despite the relative thinness of their skin—of the same order of magnitude as that reaching the larva interior in the experiments on Calliphora.

Results

1. *Calliphora erythrocephala*

Irradiation of Calliphora larvae with high X-ray doses causes rapid damage. This is evident at 2 million *r* in immediate death. Lower doses in a range of 1 million to 300,000 *r* cause death of small larvae within three days after irradiation; larger larvae succumbed within a longer time after irradiation and failed with very few exceptions to form pupae (Table 1).

Although death may be delayed, the irradiated larvae show symptoms of injury soon after irradiation; they become sluggish, ingest little or no food, and fail in consequence to show normal growth. After treatment with 300,000 *r* or more, almost all the irradiated larvae die during the larval period.

After irradiation with doses of 200,000 *r* and less a number of the irradiated larvae is still able to complete larval development and form pupae; the quantity of pupae formed increases with decreasing doses; doses of 30,000 *r* are practically without disturbing influence on the larval growth.

In general, small larvae show greater susceptibility to X-rays than large larvae (Table 1).

Puparia formed by irradiated larvae do not present the normal typically smooth and polished appearance. Their color is dull and frequently they retain original larval length, failing to shorten to the typically plump cylinder-like shape of the normal form. The time of formation of pupae from irradiated larvae as compared to the same in controls is often somewhat delayed. Although larval development may proceed to full completion following irradiation, the larva is rendered incapable of normal pupation by the rays. Pupae formed from irradiated larvae undergo liquefaction or harden. They form no mature imago.

Doses from 30,000 *r* to 5,000 *r* practically never cause injury manifest in the larval stage; pupae are formed, but are abnormal and almost incapable of forming imagos (Table 2).

TABLE 1

Calliphora erythrocephala Dose	Small larvae					Large active larvae					Larvae ready for pupation				
	I	II			III	I	II			III	I	II			III
		a	*b*	*c*			*a*	*b*	*c*			*a*	*b*	*c*	
2,000,000 *r*	10	10	0	0	0	7	7	0	0	0	7	7	0	0	0
1,000,000 *r*	10	9	1	0	0	6	0	1	5	0	7	0	7	0	0
500,000 *r*	44	32	12	0	0	20	2	4	14	0	25	5	11	9	0
400,000 *r*	33	17	15	1	0	20	0	2	18	0	17	5	5	6	1
300,000 *r*	24	9	15	0	0	17	0	0	17	0	18	2	5	10	1
200,000 *r*	34	3	13	15	3	32	2	0	16	14	24	0	3	13	8
150,000 *r*	27	1	10	9	7	19	0	0	14	5	25	5	1	5	14
100,000 *r*	29	4	5	12	8	22	0	0	9	13	19	0	2	3	14
50,000 *r*	36	2	0	17	17	26	0	0	7	19	23	0	0	1	22
30,000 *r*	27	3	0	2	22	14	0	0	3	11	6	0	0	0	6
Control	43	2	2	4	35	20	0	0	5	15	24	0	1	0	23
	317					203					195				

I—Total number of larvae in experiment.
II—Number of larvae which died in larval stage after irradiation.
a.) within 1 day.
b.) within 2-3 days.
c.) later.
III—Number of pupae formed.

TABLE 2

Calliphora erythrocephala	Small larvae				Large active larvae				Larvae ready for pupation			
Dose	I	II	III	IV	I	II	III	IV	I	II	III	IV
30,000 *r*	27	5	22	0	14	3	11	0	6	0	6	0
16,000 *r*	18	0	18	0	6	0	6	0	11	0	11	0
13,000 *r*	—	—	—	—	5	0	5	0	12	0	12	0
10,000 *r*	26	0	26	0	25	0	25	0	21	0	21	0
8,000 *r*	16	0	16	0	26	0	26	0	21	0	21	0
6,000 *r*	25	0	25	0	25	2	23	0	10	0	10	0
5,000 *r*	34	2	32	0	45	2	43	0	28	0	28	3.5
4,000 *r*	49	3	46	6.5	60	0	60	15	45	1	44	30
3,000 *r*	41	0	41	2.5	33	1	32	15	23	1	22	45
2,500 *r*	40	0	40	12.5	34	1	33	42	21	1	20	85
2,000 *r*	44	0	44	49	39	0	39	64	23	0	23	82
1,500 *r*	42	1	41	73	35	3	33	64	22	0	22	82
1,000 *r*	40	0	40	88	37	0	37	76	35	0	35	80
Control	42	0	42	93	31	0	31	77	36	0	36	89
	444				415				314			

I—Total number of larvae in experiment.
II—Number of larvae which died in larval stage.
III—Number of pupae formed.
IV—Percentage of imago formation related to number of pupae.

At doses below about 5,000 *r* an increasing percentage of irradiated larvae retains the ability to form normal pupae and to develop flies (Table 2).

Doses below about 1,000 to 1,500 *r* are practically in all cases without effect on the course of metamorphosis (Table 2). Pupation of larvae receiving these doses is normal; the pupae form flies approximately in a normal percentage of cases.

In conclusion we note that the lowest dose which produces certain inhibition of metamorphosis in irradiated Calliphora larvae (ca. 5,000 *r*) is equal to about 1/60 of the minimum dose producing certain death during the larval period in all cases, and to about 1/6 the highest dose which fails almost completely to interfere with larval development.

2. *Culex pipiens*

Whereas development of Calliphora proceeds almost normally up to pupa formation after irradiation of the larvae with doses up to 30,000 *r*, in Culex irradiation of larvae at different age period with doses above 9-11,000 *r* uniformally causes death within the larval stage (Table 3).

TABLE 3

Culex pipiens	Small larvae (first stage)					Small larvae (second stage)					Large larvae (fourth stage)				
	I	II			III	I	II			III	I	II			III
Dose		*a*	*b*	*c*			*a*	*b*	*c*			*a*	*b*	*c*	
1,000,000 *r*	10	10	0	0	0	10	10	0	0	0	7	7	0	0	0
500,000 *r*	10	10	0	0	0	10	6	2	2	0	7	7	0	0	0
250,000 *r*	10	10	0	0	0	10	7	2	1	0	7	1	6	0	0
175,000 *r*	10	10	0	0	0	11	3	3	5	0	7	0	2	5	0
100,000 *r*	10	0	0	10	0	10	2	1	7	0	8	0	1	7	0
50,000 *r*	10	0	0	10	0	11	0	1	10	0	6	0	0	6	0
25,000 *r*	11	0	0	10	1	12	0	0	12	0	7	0	0	7	0
12,000 *r*	15	0	0	15	0	10	0	0	10	0	5	0	2	3	0
11,000 *r*	15	0	0	15	0	10	0	0	10	0	6	0	1	2	3
10,000 *r*	15	0	0	15	0	10	0	0	9	1	6	0	2	2	2
Control	35	0	0	8	27	19	0	2	6	11	20	0	1	4	15
	151					123					86				

I—Total number of larvae in experiment.
II—Number of larvae which died in larval stage after irradiation.
a.) within 1 day.
b.) within 2-3 days.
c.) later.
III—Number of pupae formed.

TABLE 4

Culex pipiens	Small larvae (first stage)				Small larvae (second stage)				Medium sized larvae				Large larvae			
Dose	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
12,000 r	15	15	0	0	10	10	0	0	10	0	0	0	5	5	0	0
11,000 r	15	15	0	0	10	10	0	0	10	8	2	0	6	3	3	0
10,000 r	15	15	0	0	10	9	1	0	10	7	3	0	6	4	2	0
9,000 r	15	12	3	0	10	9	1	0	9	9	0	0	6	5	1	0
8,000 r	30	29	1	0	20	17	3	0	17	8	9	33	16	6	10	30
7,000 r	45	42	3	0	30	18	12	33	25	14	11	18	26	3	23	26
6,000 r	45	38	7	71	30	17	13	62	24	13	11	18	26	3	23	30
5,000 r	45	27	18	83	36	17	19	74	24	11	13	69	26	6	20	60
4,000 r	73	29	40	91	30	8	22	82	35	8	27	85	39	3	36	86
3,000 r	45	12	30	89	18	3	15	87	23	0	23	100	24	1	23	100
2,500 r	16	2	14	100	—	—	—	—	6	0	6	100	9	0	9	89
2,000 r	18	2	16	100	—	—	—	—	6	0	6	83	8	0	8	75
1,500 r	15	3	16	100	—	—	—	—	6	0	6	100	8	0	8	100
1,000 r	18	2	16	94	—	—	—	—	4	0	4	100	7	0	7	100
500 r	18	2	16	100	—	—	—	—	2	0	2	100	8	0	8	100
Control	48	10	38	97	20	5	15	100	22	2	20	100	24	2	22	100
	476				224				233				244			

I—Total number of larvae in experiment.
II—Number of larvae which died in larval stage.
III—Number of pupae formed.
IV—Percentage of imago formation related to number of pupae.

The time after irradiation at which death intervenes depends on the radiation dose.

Irradiation with doses of 175,000 or more *r* units causes death of the small larvae within 24 hours. In later stages of growth higher doses are necessary for rapid death. When lower doses down to 12,000 and 10,000 *r* units—the range depending on the age of the irradiated larvae—are applied, the death of the irradiated larvae occurs after a longer time.

Culex larvae like the larvae of Calliphora often give evidence of injury sustained during irradiation by feeble, jerking movements.

The results of irradiation experiments summarized in Table 4 show that irradiation with doses below 10-12,000 *r* units does not prevent the development of pupae. The number of pupae formed not only increases with decreasing doses, but these pupae develop living imagos in rapidly increasing proportion.

At doses lower than about 3,000 *r* units practically no effect on pupal development occurs.

It should be emphasized that the minimum dose causing 100 per cent fatal inhibition of development (no imagos formed) is ca. 9,000 *r* units and about 1/20 of the minimum dose causing rapid death of the larvae (ca. 175,000 *r* units).

TABLE 5

Calliphora larvae	Irradiated with	Culex larvae
Death almost in all cases in larval stage	2,000,000 *r*	Death almost in all cases in larval stage
	300,000 *r*	
Death partly in larval stage partly after pupation	30,000 *r*	
Pupae formed in almost all cases, but no formation of fly-bodies	12,000 *r*	rare pupae formation no imagines
	8,000 *r*	increasing pupae formation with frequent imago emergence
	5,000 *r*	
Increasing imago formation	3,000 *r*	
Imago formation in almost all cases	1,000 *r*	Imago formation in almost all cases

Discussion of Results and Literature

The results of the present investigation have been summarized in Table 5. Both Calliphora and Culex larvae are killed in essentially similar manner by doses above 300,000 *r*. On the other hand, the response to doses in the range of 12,000 to 200,000 *r* is different in the two species. Doses in this range impede the formation of a viable imago in both cases, but affect larval development in a manner different in each species. In Culex pipiens, all the irradiated larvae die during the larval stage and even though some moults are achieved, pupae are never formed. The response in Calliphora larvae follows a very different pattern. Irradiation of Calliphora larvae with doses of 30,000 *r* and less uniformly permits pupae formation whether irradiation is applied early or late in the larval period. When the larvae are subjected to doses over 30,000 *r*, pupation may be impeded, the proportion of successful pupation decreasing gradually with increasing doses. At about 200,000 *r* the range is reached wherein pupae formation is practically uniformly prevented. In interpreting the remarkable difference in behavior between Calliphora and Culex consideration should be given to findings in other species with regard to the effect of radiation on cell division. From findings with spermatozoa, trypanosomes and other test objects, it is known that doses which are themselves too small to cause death directly may yet impede cell division. Although cell motility may be retained in such cases, fertilization or infection cannot occur.

The interfering effects of irradiation on cell division has been demonstrated also by numerous other observations. Halberstaedter and Luntz (1929, 2), working with Eudorina elegans, have shown, for instance, that certain doses of X-rays are without visible effect on the developing colony until cell division is due. It was only at division term, the time of which was exactly predictable, that death ensued, either directly or following development of abnormal crippled forms. The significant point in these experiments on Eudorina is the finding that death occurs only at division term, independently of the time in the development cycle at which radiation is applied. It seems certain, therefore, that there exists a dose range within which irradiation does not cause immediate death or other visible damage, yet inflicts irreversible injury on the cell division apparatus. The effect of irradiation with a dose in this range becomes manifest consequently

only when division time arrives. It may be concluded therefore that also in fly larvae exposed to doses which cause mitosis injury alone, manifest effects do not occur in the larval period, because there cell division is not a vital function. Support of this interpretation is found in an investigation by Trager (1935, 8), devoted largely to larvae of Lucilia sericata of the Calliphora family. It has been shown by Trager that hypodermal tissue, fat body, salivary gland, mid-gut musculature and matrix cells of the trachea do not grow by cell division, but by increase of cell size. Mitosis occurs therefore mainly not in the larval but in the pupal stage when, the larval organs being histolyzed, imago organs develop by multiplication from islands of division—capable cells and mainly from the imaginal discs.

Larval development in Calliphora species consequently does not involve any vital cell division, histogenesis of imago organs occurring exclusively in the pupal period when cell division begins to predominate. Enzman and Haskins (1938, 1) in experiments on the delayed effect of X-rays on Drosophila arrived at conclusions similar to our own for Calliphora. These investigators, too, ascribe survival of irradiated larvae through the larval stage with subsequent onset of death only at pupation to the fact that larval cells are in a "stationary stage in the sense that most of them increase in cell size, but not in cell number." In Culicidae, according to Trager (1937, 9), a different condition prevails. For larvae of the yellow fever mosquito, Stegomya fasciata, it has been shown by Trager that certain tissues (salivary gland, gut, large oenocytes, the main tracheal tubes, gills and hair forming cells) grow by increase of cell size, but that others (hypodermal tissue, fat body and thoracic ganglia cells) grow by cell multiplication.

The tissues belonging to the latter category persist through pupation, and are ultimately integrated into the imago, whereas most of the organs growing by cell multiplication are completely broken down in the pupa. It seems likely that the existence of cell division in certain tissues of Culex larvae provides an explanation for the observation that in contrast to larvae of Calliphora those of Culex generally die in the larval stage following irradiation with a relatively small dose of X-rays (about 12,000 r).[1]

[1]A fuller report on different effects of radiation on Culex and Calliphora larvae will be published elsewhere by Hecht.

Summary

The effect of X-rays on development has been studied in larvae of Calliphora erythrocephala and Culex pipiens. In both species irradiation with sufficiently high doses causes onset of death already in the larval stage; in Calliphora, doses which produce no visible interference with larval development and pupa formation, may impede imago formation, whereas in Culex successful pupa formation after irradiation in the larval stage is followed in a large proportion of cases by an emergence of living imagos.

In Calliphora larvae, death in the larval stage is almost uniformly produced by irradiation with 300,000 *r* units or more. Increase of dose in this range causes a more rapid onset of death, especially if irradiation is carried out in an early larval stage. After irradiation with doses below 300,000 *r* units, pupa formation was found in a proportion which increased with decreasing dose. At 30,000 *r* units pupae were formed almost by all irradiated larvae. Fly-bodies were never found from pupae formed of larvae which had received doses higher than about 4-5,000 *r* units. The development of irradiated Calliphora larvae into imagos is practically unaffected by doses below 1,000 *r*.

In Culex, death in the larval stage is produced in almost all cases by irradiation with X-ray doses down to 12,000 *r* and in an early larval stage even smaller doses may be sufficient. Death occurs sooner after irradiation when high doses are applied. Irradiation with doses below 12,000 *r* permits formation of pupae in a certain percentage of cases. Following irradiation with high doses within this range, pupa formation is rare and no imagos are formed; as the dose is decreased, the percentage of pupa formed increases rapidly and the number of imagines which emerge becomes considerable. Irradiation of Culex larvae with doses of about 3,000 *r* units and less, is practically without effect on subsequent imago formation.

The suggestion is put forward that the different response to irradiation of Calliphora and Culex larvae is due to the fact that cell division is vital to larval development in Culex, but not in Calliphora.

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HETEROGONY OF OSSIFICATION IN THE HOUSE WREN[1]

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In an earlier paper (Huggins, *et al.*, 1942) data on the order and extent of ossification in the nestling house wren skeleton were presented. Continuing that study, this paper presents relative growth constants for ossification of various bones and compares these with previously determined constants for the corresponding external dimensions of the nestling wren (Huggins, 1940). Further it seemed of interest to determine whether or not there were gradients in the rate of ossification comparable to those found in the growth rates of corresponding external regions.

A. MATERIALS AND METHODS

Material used in this study was 67 nestling house wrens (Troglodytes aëdon aëdon), aged 0-14 days and although more individuals would have been desirable at least four of each age were obtained.

After the feathers had been removed the bones were stained in Alizarin Red S and specimens cleared in glycerine following the procedure of Dawson as given in Lee's *Vade Mecum* (1937). In the work on early stages the measurements of ossified areas were made by means of an ocular micrometer. In the case of the older specimens a pair of dividers and millimeter rule were used. The total lengths of bones given here do not include the outside ossification centers or heads until the epiphyses joined their respective diaphyses. Green and Fekete (1933), in a similar study on young mice, did not include data in calculations of constants until the epiphyses had joined their respective diaphyses. However, since in the case of the wren, the additions are small at any one time, so small in fact that no effect therefrom can be noted at corresponding points on the graphs, this caution seemed unnecessary. Irregularities in data are apparently

[1]A portion of this work was carried on in the Biological Laboratory, Western Reserve University, Cleveland, Ohio.

traceable to paucity of specimens rather than specific additions of bone.

Data were collected on width as well as length of developing bony areas but upon consideration of the difficulties of making this measurement from the same angle on all specimens and the relatively large error thus introduced it was decided not to make calculations of relative growth constants on this material. Some graphs were constructed, however, and although more irregular, the graphs showing width of bones at mid shaft are strikingly like the graphs for lengths of corresponding bones.

The data obtained were analyzed using the relative growth equation

$$y = bx^a.$$

The y here represents the dimension of the individual bone and the x the total length of the bird from the tip of the bill to the base of the tail. The constant a is the ratio between rate of growth of the part under consideration and the rate of growth of the whole and the constant b is the value of y when x is 1. If this formula fits the data a straight line is expected when they are plotted on double logarithmic coördinate paper, or possibly a series of straight lines. The constant a is the slope of the line and b is its y-intercept. For this study the figures for total length used are the average lengths obtained previously from about 30 living specimens of each age (Huggins, 1940). A number of additional graphs were constructed to test various theories or questions that arose but since none were particularly enlightening they are not included in this paper.

B. Results

When the various measurements of the bones are plotted with respect to time all curves are approximately sigmoid (Figure 1). The period of most rapid ossification is from the third to the eighth days in most instances. In the time graphs for external measurements the lower portion of the curve is not as sharply flattened as in the graphs of ossification, the period of rapid growth extending back to the first or second day, but the upper parts of the curves are similar, approaching their asymptotes at about the same time.

Double log graphs of the measurements plotted against total length seem to be flattened sigmoid curves in most cases (Figure 2) but for convenience they have been fitted to three straight lines. Breaks come at the third or fourth day and between the seventh and eighth

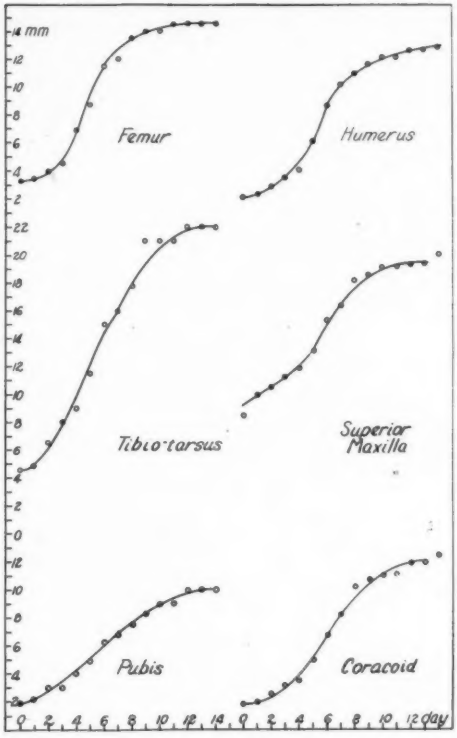

FIGURE 1
TYPICAL GROWTH CURVES OF OSSIFICATION WITH RESPECT TO TIME

days. The double log graphs of external measurements were much less obviously sigmoid yet that tendency was noted in many instances. These plots were well fitted, in most cases, by two straight lines with the break at the eighth day. In the case of the wing segments and head dimensions a single straight line seemed adequate.

The question arises as to whether or not one should compare ossification of individual bones with what may be a wholly different growth pattern, namely increase in total length. The time curve for total length is not, it is true, as much like those for ossification as for

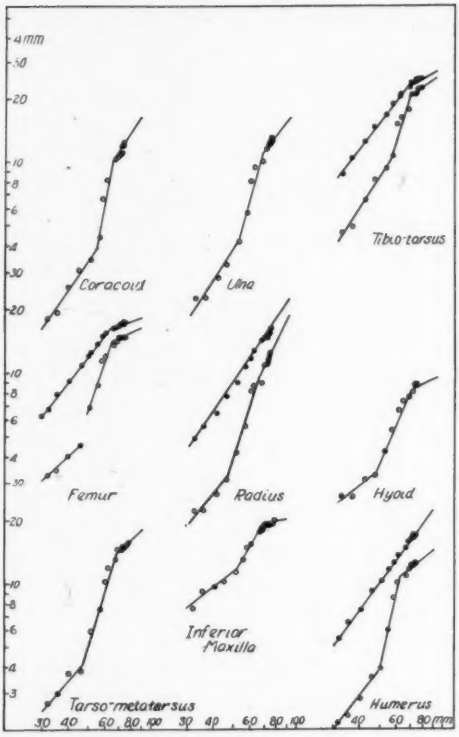

FIGURE 2

EXAMPLES OF DOUBLE LOG CURVES OF VARIOUS BONES

All are plotted with body lengths as abscissas. Curves with solid circles are for external segments corresponding to the bone represented immediately beneath.

the corresponding external segments but like them it is similar for the period from three to fourteen days and less flattened in the earliest stage. If, however, the rate of ossification of each bone is compared to that of some other bone such as the femur the resulting relative growth curves are still sigmoid in practically every case, indicating that their relationships to each other are constantly changing. No clearer picture is to be thus obtained and, moreover, it is not comparable to previous studies involving external measurements. To

TABLE 1

VALUES OF THE RELATIVE GROWTH CONSTANT α FOR OSSIFICATION OF BONES

Bone	Period				Bone (External regions)	Period	
	0-3 Days	0-4 Days	4-8 Days	5-8 Days	9-14 Days		0-8 Days	0-14 Days
Ilium		0.9528		2.4221				
Ischium	1.1132		2.0939					
Pubis	1.3088		2.4229					
Femur	0.8890		2.6166		0.1155	Thigh	1.2863	
Tibio-tarsus		1.5686		2.1606	0.3623	Shin	1.4106	
Tarso-metatarsus	1.0189		3.1254		0.2663	Shank	1.5778	
Fibula		1.3355		2.7799				
Humerus		1.3564		2.9834		Upper arm		1.3270
Radius	1.0446		3.0880			Forearm		1.4914
Ulna		1.3729		2.8097				
Third Metacarpal		1.6267				Manus		1.2150
Scapula		1.4260		2.4138				
Clavicle		1.0617		1.5903				
Coracoid		1.4935		3.7633				
Superior Maxilla		0.6314		1.7128				
Inferior Maxilla		0.7758		1.6512				
Hyoid		0.7027		2.1936				

further check on the nature of the curve of ossification, lengths of bones were plotted against head length. Since supposedly fewer growth factors are involved and the head has reached equilibrium (Huggins, 1940) it was thought that this might clarify the situation. When external measurements are plotted against head length the curves are well fitted by one or two straight lines but with ossification lengths the curves are again quite clearly sigmoid.

Table 1 gives the values of α for the first two periods mentioned above, zero or hatching day to the third or fourth day and third or fourth to eighth day. Values of α were calculated beyond the eighth day in only a few instances for it has been shown that as the time course of growth approaches its asymptote the constant of differential growth can have very little significance (Lumer, 1937). Values of the constant, α, for external measurements are included in the table for purposes of comparison. Table 2 expresses in percentages the relation between amount of ossification at the time of hatching and that attained on the fourteenth day.

TABLE 2

PERCENTAGE OF DEVELOPMENT AT HATCHING: ON THE BASIS OF DEVELOPMENT REACHED ON FOURTEENTH DAY

Bone	Per cent	Bone	Per cent
Ilium	25.4	Scapula	17.0
Ischium	20.0	Coracoid	14.0
Pubis	19.2	Clavicle	24.0
Femur	22.8	Humerus	16.9
Tibio-tarsus	20.7	Radius	18.0
Fibula	24.0	Ulna	16.7
Tarso-metatarsus	16.8	Third Metacarpal	16.8
First Metatarsal (remanent)	14.5	Second Digit	7.4
First Toe, First Phalanx	14.1	Third Digit, First Phalanx	15.5
First Toe, Nail	14.7	Third Digit, Second Phalanx	13.3
Second Toe, First Phalanx	8.8	Fourth Digit	0
Second Toe, Second Phalanx	12.5	Superior Maxilla	42.5
Second Toe, Nail	17.1	Inferior Maxilla	37.5
Third Toe, First Phalanx	13.0	Hyoid	28.9
Third Toe, Second Phalanx	14.4		
Third Toe, Third Phalanx	13.0		
Third Toe, Nail	14.8		
Fourth Toe, Second Phalanx	8.1		
Fourth Toe, Third Phalanx	7.3		
Fourth Toe, Fourth Phalanx	10.1		
Fourth Toe, Nail	13.5		

C. Discussion

Hasty consideration of Table 1 seems to indicate a haphazard pattern of ossification without definite gradients. However the percentage of development (Table 2) shows that at the time of hatching the ossification of the femur is nearer completion than the tibio-tarsus which in turn is further developed than the tarso-metatarsus. Thus indicating that during the pre-hatching period a proximo-distal gradient must have existed. In general the small bones from this point out to the ends of the toes must also have been included in this gradient. The reversal of the gradient in the ends of the toes may be accounted for on the basis of relative incompleteness of ossification of these bones and even, in the case of the nails, of external growth by the time the nestling is fourteen days old. For example, the nail of the first toe has reached only eighty per cent of its adult length on the fourteenth day while the external size of the rest of this toe is practically the same as that of the adult (Huggins, 1940). The fibula will be noted as an exception to the above scheme. This would seem to be because the fibula of birds is much reduced from its comparative importance in more primitive or less specialized vertebrates, being thus reduced seems to be a matter of complete or nearly complete cessation of development at some definite point rather than of a generalized retardation extending over a long period. The time curve for the ossification of the fibula is very similar to that of the tibio-tarsus up to the eighth day but from the eighth to the twelfth day there is absolute cessation of growth. On the last two observed days there was a very small increase in the length of the ossified area. It has, therefore, reached a higher per cent of its ultimate development at the time of hatching because its ultimate goal has been modified. Hersh (1941) refers to such parts as "centers of defective growth."

The ilium fits into this leg gradient on the basis of percentage of development, being slightly further developed than the femur (25.4 per cent as compared with 22.8 per cent). Thus it would appear that the center of greatest ossification was close to the body in the pre-hatching period. During the next phase reference to Table 1 will show that this principal growth center has passed distally to the tibio-tarsus and in the next period moves distally to the tarso-metatarsus but the gradient is not regular out to the tips of the toes.

A dorso-ventral gradient is evidenced by the pelvic girdle on the basis of percentage of development at hatching: Ilium, 25.4; Ischium, 20.0; and Pubis, 19.21, and there is a ventro-dorsal gradient from hatching to the fourth day as shown by the respective values of α. No gradient is apparent in the next period.

In the case of external growth of the wren (Huggins, 1940) a postero-anterior gradient was found along the main body axis but the relative rates for the paired appendages did not fit into this scheme nor does the relative rate of ossification or percentage of development fit into such an arrangement. In the case of the Backswimmer, Clark and Hersh (1939) noted that there was a postero-anterior gradient in the tibias of the three pairs of legs. The only instance of a similar gradient among vertebrates that the authors have been able to find is in the work of Green and Fekete (1933). There it will be noted that at birth the humerus of the mouse has reached 26 per cent of its ultimate size and the femur only 17 percent indicating an antero-posterior gradient prior to birth. Subsequently there is a postero-anterior gradient as evidenced by the values of α, that of the humerus being 0.89761 while that of the femur is 1.18946. One possible method of accounting for this difference is the relative lack of specialization, "primitiveness," of the limbs of the mouse as compared with those of the wren, particularly the forelimb. The wing is probably developed for flight as a secondary habit, modifications being in the nature of additions superimposed on the primitive growth pattern "late in life." Green and Fekete also found a similarity between the gradients in the fore and hind limbs of the mouse though neither external measurements nor bone dimensions of the wren show such a gradient in the wing either before or after hatching. The suggestion given above might very well be applied in this case also. Similarly the lack of definite gradient, such as is found in the pelvic girdle, for the pectoral girdle may be due to the late development of the habit of flight and its accompanying derangement of the growth pattern.

No exact measurements were made on vertebrae or ribs but in the case of the vertebrae a qualitative gradient was recorded. On the day of hatching the vertebrae of the cervical and thoracic region were observed to be well developed, lumbar and sacral vertebrae are barely visible, and some coccygeal vertebrae are not yet distinguish-

able on the second day (Huggins *et al.*, 1942). This is not a perfect gradient as thoracic vertebrae are more heavily ossified at the time of hatching than the cervical. The center rib seems to develop first with amount of growth diminishing in an orderly way in both directions. The sternum is not present at the time of hatching which might be interpreted as a manifestation of a dorso-ventral gradient.

The double log graphs for ossification plotted against body seems to be rather definitely sigmoid as has been mentioned above. This tendency is much less apparent in plots of corresponding external measurements but if the length of ossified areas are graphed against corresponding external dimensions on a double log grid this tendency is even more marked. This is particularly clear in the case of Femur-Thigh and Humerus-Upper-arm graphs (Figure 3). Clark and Hersh (1939) have pointed out that if the relative growth equation is a true approximation for the relationship of a leg segment it cannot be a true approximation for the leg as a whole. In view of this it might be suggested that if the relative growth equation is a true approximation for the growth of a limb segment it could not be a true approximation of any measurement of internal change which is not in direct proportion to the external growth.

D. Summary

The time course of increase in length of ossified areas is sigmoid with the period of most rapid ossification from the third to the eighth day. The fibula follows the same pattern to the eighth day but stops abruptly on that day.

When double logarithmic graphs of bone length plotted against total length are constructed, flattened sigmoid curves result but for convenience they are fitted to three straight lines.

Percentage of ultimate development attained at hatching have been calculated. These indicate a proximo-distal gradient during the pre-hatching period. Immediately after hatching, the center of greatest ossification as shown by values of α, passes to the tibio-tarsus and in the next period moves distally to the tarso-metatarsus. The bones of the pelvic girdle present a dorso-ventral gradient at hatching which is reversed from hatching to the eighth day. No gradients are present in the wings.

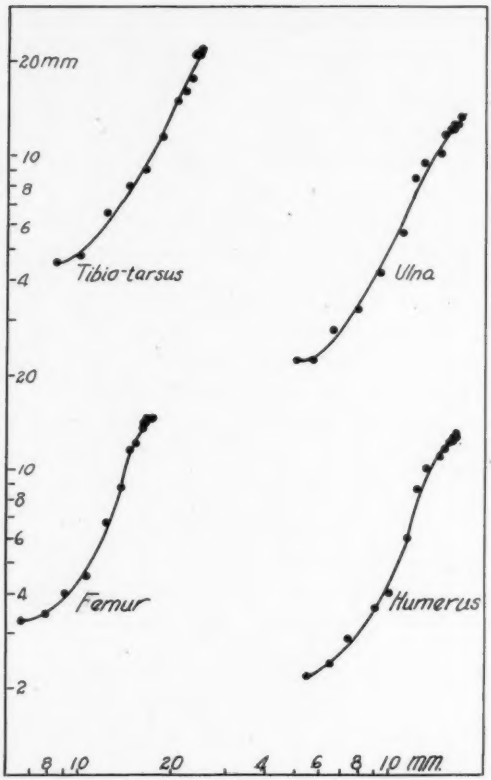

FIGURE 3

SOME ILLUSTRATIONS OF CURVES OBTAINED WHEN BONE LENGTH (y AXIS) ARE PLOTTED AGAINST CORRESPONDING EXTERNAL SEGMENTS (x AXIS)

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LENGTH-GROWTH CURVES FOR THE RAZOR CLAM

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INTRODUCTION

Weymouth, McMillin, and Rich (1931) have presented extensive data on the growth and development of the razor clam (Siliqua patula). They sought, among other things, to find a mathematical formula that would represent the average length of the razor clam at the end of each year. No single formula was found that would satisfactorily represent the growth in length of the razor clam. The purpose of this note is to give a single formula that will represent the linear growth of clams and to call attention to some aspects of "fitting" such a formula that have not been stressed previously.

The same method of fitting has given satisfactory results with a great variety of other data. One great advantage of the proposed method is that it can be applied to partial data and extrapolations made to a certain extent without serious error.

GENERALIZED GROWTH CURVE

A growth curve such as that shown in Figure 1 strongly suggests a cumulative frequency distribution. Also, the basic idea of accumulation is present in both cases. The first derived curve is, thus, similar to an ordinary frequency distribution and could perhaps be handled in the same way.

Two widely used "laws of growth" of linear dimensions are

(1) $$L = at^b$$

and

(2) $$L = A(1 - e^{-kt})$$

where L is linear dimension, t is time and a, b, A, and k are constants. In both cases the first derivative of L with respect to time satisfies a differential equation of the form

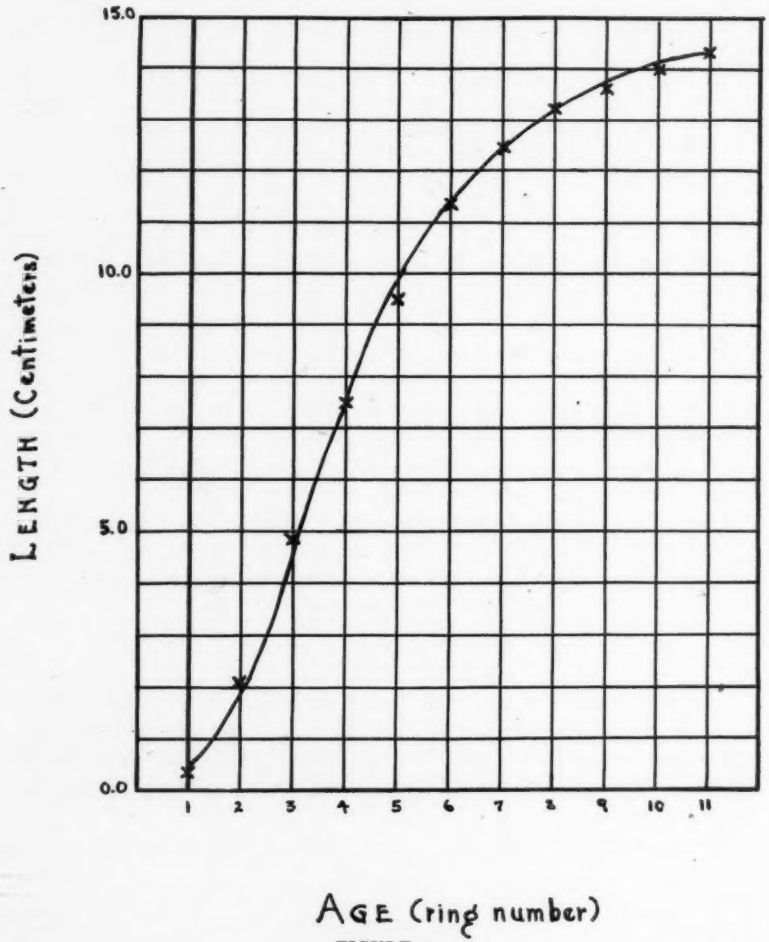

FIGURE 1

SMOOTH LENGTH-GROWTH CURVE AND OBSERVED AVERAGE LENGTHS AT YEARLY AGES FOR 16 ELEVEN-YEAR-OLD CLAMS

$$\frac{dy}{dx} = \frac{y(a - x)}{b_0 + b_1 + b_2x^2} \tag{3}$$

We shall take tentatively (3) as a sufficiently broad basis of the growth process. Equation (3) is also the basis of the well known Karl Pearson's system of frequency distributions. Methods of fitting have been worked out both when the observed distribution is complete, Elderton (1927), and when only a stump or portion of a distribution is available, Carver (1924).

There is a roughness about observed growth data that has usually been attributed to random sampling variations but is due essentially to the effect of environment on the growing organism. Thus in finding a growth curve for the length of clams we would presumably be interested in a "law" which is free of environmental variations. Now in nature there are undoubtedly good-clam years and bad-clam years. These may occur more or less cyclically or in some other manner. These are conditions that tend to affect all clams alike and will make any observed growth data somewhat erratic no matter how many individuals are observed. The precise effect of varying environment cannot be estimated very well on the basis of present information. However, it is indicated that some liberties can justifiably be taken with the observed data in fitting a mathematical formula. A least-square or other conventional fitting of a curve to the observed data will not necessarily give a "best" or even a "good" representation of the data. These remarks will be extended and illustrated in the following example.

Example

As an example we shall consider the length-growth curve of 16 eleven-year-old clams, the data for which were furnished by Professor F. W. Weymouth of Stanford University. The average lengths of these clams at the end of successive years were 0.37 (4 measurements), 2.06, 4.82, 7.48, 9.48, 11.36, 12.44, 13.22, 13.59, 14.02, 14.34 centimeters. The first differences were taken and plotted at the midpoints of the yearly intervals and subjected to a French-curve smoothing. The differences so obtained are 0.42, 1.65, 2.76, 2.66, 2.07, 1.50, 1.10, 0.75, 0.50, 0.34.

The smoothed differences were graduated by the formula

$y_{x+1}/y_x = (x^2 - 5.9550x + 13.7955)/(x^2 - 3.4525x + 5.7440)$
where y_{x+1} and y_x are successive ordinates of the first-difference curve. This formula is obtained by difference-equation graduation, Carver (1924). The calculated ordinates of the first-derived curve are, then, 0.48, 1.28, 2.66, 2.99, 2.25, 1.51, 1.01, 0.70, 0.50, 0.37. These ordinates are plotted, a smoothed curve is drawn through them, and a planimeter is used to read off the length corresponding to each year of age. The total area is adjusted so that size 14.34 is reached at the end of 11 years. A slight adjustment is necessary for the first year so that the size is zero when the time is zero. A straight line was used from the point (0,0) to the point (½,0.48), time being the abscissa. The calculated lengths at the end of the successive years are 0.46, 1.78, 4.47, 7.54, 9.82, 11.36, 12.39, 13.14, 13.67, 14.03, 14.34.

It should be noted that the final step could be accomplished by converting the graduating formula for the first derived curve to the corresponding Pearson curve and integrating between proper limits. In some cases tables are available so that this can be done fairly easily.

The smoothed and observed laws of growth for these 16 eleven-year-old clams are presented in Figure 1. The smoothed curve is a "good" representation of the observed data but is not a "best" representation in any sense of the word.

Summary

Present growth curves are generalized by generalizing the differential equation which the first-derivatives of such curves satisfy. When this is done the generalized differential equation is found to be identical with the equation which is the basis for Pearson's system of frequency distributions. "Good" methods of fitting such distributions to observed data have already been worked out.

Least-square technique and other conventional methods of fitting do not necessarily give good results because of the effects of environment on growth rates from year to year. Because of the lack of the extremely extensive information that is necessary for any useful estimate of environmental effects a preliminary French-curve smoothing of the observed data was resorted to for a "good" representation of the observed data by a smooth mathematical curve. On this basis a single curve has been obtained which gives a "good" representation

of the growth in length of a particular group of clams but which does not give a "best" representation. A "best" representation in any conventional sense does not seem possible when the observed data are collected under uncontrolled environmental conditions.

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